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(54) Title: ALPHAVIRUS VECTORS AND VIROSOMES WITH MODIFIED HIV GENES FOR USE AS VACCINES

(57) Abstract: The present invention provides methods and compositions comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit integrase, RNase H and/or reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.

**ALPHAVIRUS VECTORS AND VIROSOMES
WITH MODIFIED HIV GENES FOR USE AS VACCINES**

This application claims priority to U.S. Provisional Application No. 60/216,995,
5 filed July 7, 2000 which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

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The present invention relates to vaccines using viral antigens, and in particular, to vaccines for the treatment and prevention of human immunodeficiency virus (HIV) infection. The vaccines of this invention comprise alphavirus RNA replicon systems which contain nucleic acid sequence encoding antigens for eliciting an immune response to HIV.

15

Background

The successful control of the AIDS epidemic will require an effective vaccine
20 for human immunodeficiency virus type 1 (HIV) that significantly reduces or prevents the spread of infection. Currently, several viral vector systems as well as naked DNA are at various stages of pre-clinical and clinical evaluation as candidate HIV vaccines. Recombinant poxviruses are the most widely studied virus vectors and are furthest along in clinical development (e.g., ALVAC).

25

The alphavirus-based replicon particle systems, such as the ones described in U.S. Patent No. 5,792,462 and herein referred to as "VRPs," have multiple distinct properties that make them attractive as an HIV vaccine delivery technology. These properties include: natural targeting to and expression in lymphoid tissues (an optimal site for induction of an immune response); high antigen expression levels, e.g., up to 30 20% of total cell protein; induction of balanced humoral, cellular, and mucosal immune responses; sustained efficacy over multiple simultaneous or sequential inoculations of

the vector; and a high margin of safety.

Venezuelan equine encephalitis virus (VEE) is a member of the Alphaviruses group, which also includes the prototype Sindbis virus (SIN) and Semliki Forest virus (SFV), and is comprised of enveloped viruses containing plus-stranded RNA genomes within icosahedral capsids (Strauss, 1994). Alphavirus genomes are: approximately 11.5 kb long, capped, polyadenylated, and infectious under appropriate transfection conditions. The nucleocapsid is composed of 240 molecules of the capsid protein arranged as a T=4 icosahedron, and is surrounded by a lipoprotein envelope (Paredes *et al.*, 1993). Protruding from the virion surface are 80 glycoprotein spikes, each of which is a trimer of virally encoded E1 and E2 glycoprotein heterodimers. The virions contain no host proteins.

Alphaviruses share replication strategies and genomic organization. The complete replicative cycle of alphaviruses occurs in the cytoplasm of infected cells. Expression from the alphavirus genome is segregated into two regions. The four enzymatic nonstructural proteins (nsP1-nsP4) are synthesized from the 5' two-thirds of the genome-length RNA and are required for RNA replication. Immediately following infection, the nsPs are produced by translation of parental genomes and catalyze the synthesis of a full-length negative-sense copy of the genome. This serves as a template for the synthesis of progeny plus-stranded genomes.

The negative-sense copy of the genome also serves as the template for the synthesis of subgenomic mRNA at approximately 10-fold molar excess relative to genomic RNA in infected cells (Schlesinger and Schlesinger, 1990). Synthesis of subgenomic 26S mRNA is initiated from the highly active internal 26S mRNA promoter, which is functional only on the negative-sense RNA. The subgenomic mRNA corresponds to the 3' one-third of the genome and encodes the alphavirus structural proteins.

- Full-length, infectious cDNA clones of the RNA genome of VEE (Davis *et al.*, 1989) have been constructed, a panel of mutations which strongly attenuate the virus have been identified (Johnston and Smith, 1988; Davis *et al.*, 1990), and various constellations of these attenuating mutations have been inserted into the clones to 5 generate several live attenuated VEE vaccine candidates (Davis *et al.*, 1991; 1995b; Grieder *et al.*, 1995). The resulting vaccine candidates are avirulent and provide complete protection against lethal virus challenge in rodents, horses and nonhuman primates.
- 10 The alphavirus VRPs are propagation defective, single cycle vectors that contain a self-amplifying alphavirus RNA (replicon RNA) in which the structural protein genes of the virus are replaced by a heterologous antigen gene to be expressed. Alphavirus VRPs are typically made in cultured cells, referred to as packaging cells. Following introduction into mammalian cells, the replicon RNA is packaged into VRP 15 by supplying the structural proteins in "trans," i.e. the cells are co-transfected with both replicon RNA and one or more separate helper RNAs which together encode the full complement of alphavirus structural proteins. Importantly, only the replicon RNA is packaged into VRP, as the helper RNA(s) lack the *cis*-acting packaging sequence required for encapsidation. Thus, the VRPs are defective, in that they can only infect 20 target cells in culture or *in vivo*, where they express the heterologous antigen gene to high level, but they lack critical portions of the VEE genome (i.e., the VEE structural protein genes) necessary to produce virus particles which could spread to other cells.

Delivery of the replicon RNA into target cells (for vaccination) is facilitated by 25 the VRP following infection of the target cells. In the cytoplasm of the target cell, the replicon RNA is first translated to produce the viral replicase proteins necessary to initiate self-amplification and expression. The heterologous antigen gene is encoded by a subgenomic mRNA, abundantly transcribed from the replicon RNA, leading to high level expression of the heterologous antigen gene product. Since the VEE structural 30 protein genes are not encoded by the replicon RNA delivered to the target cell, progeny

- virion particles are not assembled, thus limiting the replication to a single cycle within the infected target cell. Experimental VRP vaccines have been successful in vaccinating rodents against influenza virus, Lassa fever virus and Marburg virus (Pushko *et al.*, 1997; Hevey *et al.*, 1998). In nonhuman primates, VRP vaccines have
- 5 demonstrated complete efficacy against lethal Marburg virus challenge (Hevey *et al.*, 1998), shown partial but significant protection against SIV infection and disease (Davis *et al.*, 2000) and induced an anti-HA response at a level consistent with protection of humans against influenza virus infection.
- 10 The alphavirus based replicon vector systems, and in particular the VEE-based systems, present several advantages in vaccination, including safety and high immunogenicity/efficacy. VEE is unique among the alphaviruses in that a live attenuated IND VEE vaccine, TC-83, (Kinney *et al.*, 1989; Kinney *et al.*, 1993) has been inoculated into approximately 8,000 humans. This allows direct safety and
- 15 efficacy comparisons between human, nonhuman primate and rodent responses to the same VEE derivative. A large body of experience strongly suggests that the animal models generally reflect the human susceptibility and disease course, except that mice are far more susceptible to lethal VEE disease than humans or nonhuman primates. Furthermore, the VEE replicon vectors express high levels of the gene of interest in cell
- 20 culture, and *in vivo* expression is targeted to lymphoid tissues, reflecting the natural tropism mediated by the VEE glycoproteins. Cells in the draining lymph node of VRP-inoculated mice contain detectable amounts of the desired gene product within hours of inoculation. This expression continues for up to five days.
- 25 To date, VRP vector vaccines have been used in over 2000 rodents and in 94 macaques at doses up to 5×10^8 i.u., with no indication of any clinical manifestations.

In work reported by Pushko *et al.* (1997), individual mice were immunized sequentially with Lassa virus N-VRP and influenza virus HA-VRP. Groups of mice, 30 which received two inoculations of 3×10^4 or 3×10^6 i.u. of Lassa N-VRP followed by

two inoculations of 2×10^5 i.u. of HA-VRP, all responded with serum antibodies to both antigens. The level of anti-influenza antibody induced in these sequentially inoculated mice was equivalent to a control group, which received two inoculations of buffer followed by two inoculations of 2×10^5 i.u. of HA-VRP. All HA-VRP
5 immunized mice were completely protected against influenza virus challenge. Furthermore, sequential immunization of mice with two inoculations of N-VRP prior to two inoculations of HA-VRP induced an immune response to both HA and N equivalent to immunization with either VRP construct alone. Primary and booster immunization with a VRP preparation expressing an immunogen from one pathogen
10 did not interfere with the development of a protective response to subsequent primary immunization and boosting with VRP expressing an immunogen from a second pathogen, thus showing that the VRP-based system can be used to induce immunity to a variety of pathogens in the same individual over time.

15 Four macaques were inoculated subcutaneously at week 0 with 10^5 i.u. each of SIV-gp160-VRP (*env*) and SIV MA/CA-VRP (*gag*), boosted by the same route at week 7 with 10^7 i.u. of each VRP vaccine, and intravenously at weeks 12 and 20 with 5×10^8 i.u. of each VRP. Two control animals were inoculated with equivalent doses of HA-VRP (haemagglutinin, a glycoprotein from influenza virus), and two with the vehicle
20 only. The four SIV-VRPs immunized monkeys received subcutaneously an additional dose of 2×10^7 i.u. of gp140-VRP at week 41, followed by a final boost of 2×10^7 i.u. each of gp140-VRP and MA/CA-VRP at week 49. Four weeks after the final immunization, all eight macaques were challenged intravenously with the pathogenic virus, SIVsmE660.

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After these inoculations, three of four test macaques had measurable CTL-specific killing directed against both SIV *gag* and *env*, all four had gp160 IgG antibody by ELISA, and the three animals which harbored SIV-specific CTL also showed neutralizing antibody to SIVsmH-4.

30

Four of four vaccinated animals were protected against disease for at least 16 months following intravenous challenge with the pathogenic SIV swarm, while the two vehicle controls required euthanasia at week 10 and week 11, post challenge. In two of the vaccinees, plasma virus levels were below the limit of detection by branched chain 5 DNA assay. At 64 weeks post challenge, all four vaccinated animals showed no clinical signs of disease. One animal remained vDNA negative at 64 weeks.

The results of this highly pathogenic challenge demonstrated that the immune response induced by vaccination with SIV-VRP was effective in preventing early 10 mortality and increasing the ability to suppress challenge virus replication. The ability to control SIV replication and reduce viral load to undetectable levels was closely correlated with the strongest measurable antibody and cellular immune responses.

While these results are encouraging, the level of protection obtained would not 15 be acceptable for a human vaccine against HIV infection. Thus, there remains a need for a robust, effective and safe vaccine against HIV infection in humans. Development of a HIV vaccine comprising the complete, or immunogenic fragments of the, *gag* gene (Gag-VRP), an immunogenic portion of the *pol* gene (Pol-VRP), and the complete, or immunogenic fragments of the, *env* gene (Env-VRP), would increase the diversity of 20 available CTL epitopes substantially and thus address this need.

SUMMARY OF THE INVENTION

The present invention provides a composition comprising two or more isolated 25 nucleic acids selected from the group consisting of an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles 30 containing the *gag* gene product or the immunogenic fragment thereof and their release

from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity.

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Also provided is a composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.

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In addition, the present invention provides a composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse

transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle, and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

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10. A method of making a population of alphavirus replicon particles of this invention is provided herein, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

10 (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

15 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

20 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

25 wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

30 (b) producing the alphavirus particles in the helper cell; and

- (c) collecting the alphavirus particles from the helper cells;
- B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particles, comprising in an alphavirus-
5 permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- 10 and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- 15 wherein the combined expression of the alphavirus replicon RNA and the helper RNA produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;
- 20 (b) producing the alphavirus particles in the helper cell; and
- 25 (c) collecting the alphavirus particles from the helper cells;

- C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;
- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and
- D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second

helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles.

Also provided is a method of making a population of alphavirus replicon
5 particles, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is 25 unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

30 (b) producing the alphavirus particles in the helper cell; and

- (c) collecting the alphavirus particles from the helper cells;
- B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particle, comprising in an alphavirus-
5 permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or
10 immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein;
15 and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein
20 not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
wherein the combined expression of the alphavirus replicon RNA and the helper
25 RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or
30 more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells;
- C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells; and
- D) combining the first population of alphavirus particles produced from the first
5 helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles.

Furthermore, the present invention provides a composition comprising two or
10 more isolated nucleic acids selected from the group consisting of an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of
15 virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse
20 transcriptase functions in the *pol* gene product or immunogenic fragment thereof.

In addition, the present invention provides a composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus,
25 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release
30 from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an

immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are
5 each contained within a separate alphavirus replicon particle.

Also provided herein is a composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an
10 10 immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell,
15 and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each
20 contained within a separate alphavirus replicon particle, and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

In these embodiments, the *gag* gene product or immunogenic fragment thereof
25 can be modified by mutation of the second codon, whereby a glycine is changed to an alanine and the *pol* gene product or immunogenic fragment thereof can be modified by mutation of the nucleotide sequence encoding the active site motif, whereby YMDD is changed to YMAA or HMAA. In addition, the *pol* gene product or immunogenic fragment thereof is modified to produce only p51 of the *pol* gene product or
30 immunogenic fragment thereof.

The present invention provides a method of making a population of alphavirus replicon particles, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

5 (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

10 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

15 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

20 wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

25 (b) producing the alphavirus particles in the helper cell; and
(c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- 5 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- 10 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- 15 and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper
20 RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;
- 25 (b) producing the alphavirus particles in the helper cell; and
(c) collecting the alphavirus particles from the helper cells;
- C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- 30 (i) an alphavirus replicon RNA, wherein the replicon RNA

comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or
5 inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and
10 furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein
15 not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;
wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is
20 unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

(b) producing the alphavirus particles in the helper cell; and
(c) collecting the alphavirus particles from the helper cells; and
25

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles.

An additional method of making a population of alphavirus replicon particles is provided, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

5 (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

10 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

15 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

20 wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said

25 first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

30 B) (a) providing a second helper cell for producing a second population of

infectious, replication defective alphavirus particle, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;
- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells;

- C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;
- 30 (b) producing the alphavirus particles in the helper cell; and

- (c) collecting the alphavirus particles from the helper cells; and
- D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles.

In each of the methods above, the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell can comprise

10 sequence encoding at least one alphavirus structural protein and the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, can encode at least one other alphavirus structural protein not encoded by the replicon RNA.

15 Furthermore, in the methods above which recite attenuating mutations, only at least one of the first population of alphavirus particles, the second population of alphavirus particles and the third population of alphavirus particles can comprise particles wherein at least one of the replicon RNA, the first helper RNA, and the one or more additional helper RNA(s) comprises one or more attenuating mutations.

20

The present invention further provides alphavirus particles produced by any of the methods of this invention.

The present invention further provides a method of inducing an immune

25 response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the populations and/or compositions of this invention, in a pharmaceutically acceptable carrier.

Also provided herein is a method of treating or preventing infection by human

30 immunodeficiency virus in a subject, comprising administering to the subject an

immunogenic amount of the populations and/or compositions of this invention, in a pharmaceutically acceptable carrier.

- Also provided by the present invention is an alphavirus replicon virosome
- 5 comprising an alphavirus replicon RNA encapsidated by a lipid bilayer comprising alphavirus glycoproteins, E1 and E2, which in one embodiment, can be Venezuelan Equine Encephalitis glycoproteins E1 and E2.

- A method of producing an alphavirus replicon virosome is further provided,
- 10 comprising: a) combining alphavirus replicon RNA, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and b) gradually removing detergent, whereby alphavirus replicon virosomes are produced. Also provided is a virosome produced by this method.

- 15 Furthermore, the present invention provides a method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon virosome of this invention in a pharmaceutically acceptable carrier.

- 20 The present invention additionally provides a method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon virosome of this invention, wherein the virosome comprises alphavirus replicon RNA encoding one or more HIV immunogens.

- 25 In further embodiments, the present invention provides a composition a population of alphavirus replicon virosomes comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus,
- 30 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment

- thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon virosome.

10

- Additionally provided herein is a composition comprising a population of alphavirus replicon virosomes comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation of reverse transcriptase activity in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon virosome.

25

- A method of producing a population of alphavirus replicon virosomes is provided herein, comprising:
- A) (a) producing a first population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *env* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-

cationic lipids and detergent; and

- b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

5

- B) (a) producing a second population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *gag* gene product or immunogenic fragment thereof, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

- b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

15

- C) (a) producing a third population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding the *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

- b) gradually removing detergent, whereby alphavirus replicon virosomes are produced; and

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- D) combining the first population of alphavirus replicon virosomes, the second population of alphavirus replicon virosomes and the third population of alphavirus replicon virosomes to produce the population of alphavirus replicon virosomes.

30

In addition, a method of producing a population of alphavirus replicon virosomes is provided, comprising:

- A) (a) producing a first population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *env* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and
- 5 b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

10

- B) (a) producing a second population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *gag* gene product or immunogenic fragment thereof, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles
- 15 containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and
- b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

20

- C) (a) producing a third population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding the *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation of
- 25 reverse transcriptase activity in the *pol* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and
- b) gradually removing detergent, whereby alphavirus replicon virosomes are produced; and
- D) combining the first population of alphavirus replicon virosomes, the second
- 30 population of alphavirus replicon virosomes and the third population of alphavirus

replicon virosomes to produce the population of alphavirus replicon virosomes of claim 48.

- Furthermore, the present invention provides a method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of the virosomes of this invention, in a pharmaceutically acceptable carrier.

- Also provided is a method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the virosomes of this invention, in a pharmaceutically acceptable carrier.

- Additionally provided by this invention is a composition comprising heparin affinity-purified alphavirus replicon particles, wherein the alphavirus replicon particles comprise at least one structural protein which comprises one or more attenuating mutations, as well as a method of preparing heparin affinity-purified alphavirus particles, comprising:

- a) producing alphavirus replicon particles, wherein the alphavirus replicon particles comprise at least one structural protein which comprises one or more attenuating mutations;
- b) loading the alphavirus replicon particles of step (a) in a heparin affinity chromatography column; and
- c) collecting the fraction from the column which contains the heparin affinity-purified alphavirus replicon particles.

25

In further embodiments, the present invention provides a method of producing VRP for use in a vaccine comprising:

- a) producing a plasmid encoding the nucleotide sequence of an alphavirus replicon RNA;
- 30 b) producing a plasmid encoding the nucleotide sequence of one or more

helper RNAs;

c) transcribing the plasmids of steps (a) and (b) into RNA *in vitro*;

d) electroporating the RNA of step (c) into a Vero cell line; and

5

e) purifying VRP from the Vero cell line of step (d) by heparin affinity chromatography. By this method, VRPs can be produced in large-scale.

In additional embodiments, the present invention provides an isolated nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof. This nucleic acid can be present in a composition and in a vector.

10 Such a vector can be present in a cell. This nucleic acid can also be present in an alphavirus replicon particle.

15

The present invention further provides a method of making an alphavirus replicon particle comprising nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, comprising

20

a) providing a helper cell for producing an infectious, defective alphavirus particle, comprising in an alphavirus-permissive cell:

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(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or

30

inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

- 5 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- 10 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

15 and with at least one of said helper RNAs lacking an alphavirus packaging signal;

20 wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- 25 (b) producing the alphavirus particles in the helper cell; and
- 30 (c) collecting the alphavirus particles from the helper cell.

In the method described above, at least one of the replicon RNA, the first helper RNA, and the one or more additional helper RNA(s) can comprises one or more attenuating mutations. The present invention additionally provides alphavirus replicon particle produced according to the above methods.

Further provided is a method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of a composition comprising alphavirus replicon particles encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol*

gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof in a pharmaceutically acceptable carrier.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. DNA plasmid map of VEE replicon RNA expressing the HIV *gag* gene (p3-40.1.6). The plasmid is 12523 base pairs in length and encodes a single polyprotein expressing the four non-structural genes nsP1-4, the Clade C *gag* gene and antibiotic resistance marker, Kanamycin KN(R). The plasmid contains two promoter regions, the T7 polymerase promoter and the 26S RNA promoter. The unique NotI restriction enzyme site used to linearize prior to *in vitro* transcription is also noted.

Figure 2. DNA plasmid map of the capsid helper construct (p3-13.2.2). The plasmid is 5076 base pairs in length and encodes the VEE capsid gene (C) and antibiotic resistance marker, Kanamycin KN(R). The plasmid contains two promoter regions, the T7 polymerase promoter and the 26S RNA promoter. The unique NotI restriction enzyme site used to linearize prior to *in vitro* transcription is also noted.

20

Figure 3. DNA plasmid map of the glycoprotein helper construct (p3-13.4.6). The plasmid is 6989 base pairs in length and encodes the VEE glycoprotein genes (E3, E2, 6K and E1) and antibiotic resistance marker, Kanamycin KN(R). The plasmid contains two promoter regions, the T7 polymerase promoter and the 26S RNA promoter. The unique NotI restriction enzyme site used to linearize prior to *in vitro* transcription is also noted.

Figure 4. DNA plasmid map of VEE replicon RNA expressing HIV *pol* (p51) gene (p13-60.2.14). The plasmid is 12379 base pairs in length and encodes a single polyprotein expressing the four non-structural genes, nsP1-4, the Clade C *pol* (p51)

gene and antibiotic resistance marker, Kanamycin KN(R). The plasmid contains two promoter regions, the T7 polymerase promoter and the 26S RNA promoter. The unique NotI restriction enzyme site used to linearize prior to *in vitro* transcription is also noted.

5

Figure 5. DNA plasmid map of VEE replicon RNA expressing HIV *env* gene (pERK-DU151env). The plasmid is 13584 base pairs in length and encodes a single polyprotein expressing the four non-structural genes, nsP1-4, the Clade C env gene and antibiotic resistance marker, Kanamycin KN(R). The plasmid contains two promoter regions, the T7 polymerase promoter and the 26S RNA promoter. The unique NotI restriction enzyme site used to linearize prior to *in vitro* transcription is also noted.

Figure 6. Western immunoblot, demonstrating the expression of HIV proteins in baby hamster kidney (BHK) cells infected with VRPs. The outer lanes of the panel 15 are standard molecular weight markers. Lane 1 is the expression from VRPs encoding the p51 (*pol*) gene. Lane 2 is the expression from VRPs encoding the GP-160 (*env*) gene. Lane 3 is the expression from VRPs encoding the p55 (*gag*) gene. Arrows indicate proteins migrating with the apparent molecular weight of each respective protein.

20

Figure 7. Western immunoblot of cells infected with the Du151*env* VRP. At 18 hr post infection, the cells were lysed and the lysate run in a denaturing polyacrylamide gel. Proteins were transferred out of the gel onto a filter and the filter was probed with serum from subject Du151 using Western immunoblot methods. Lane 25 1, uninfected U87.CD4-CXCR4 cells. Lane 2, uninfected U87.CD4-CCR5 cells. Lane 3, infection of a mixed culture of U87.CD4-CXCR4 cells and BHK cells (mixtures were used as a positive control in case the U87 cells were refractory to infection by the VRP, which did not turn out to be the case). Lane 4, infected U87.CD4-CXCR4 cells. Lane 5, infected BHK cells. Lane 6, infection of a mixture of BHK cells and 30 U87.CD4-CCR5 cells. Lane 7, infected U87.CD4-CCR5 cells. The positions of

molecular weight of markers run in the same gel are shown on the right, and the inferred positions of gp160 and gp120 are shown on the left.

- Figure 8. Micrographs of U87.CD4-CCR and BHK cells used to examine expression and syncytium formation of DU151 envelope expressed from the VEE replicon. U87.CD4-CCR5 cells alone (panel 1), or a mixture of U87.CD4-CCR5 and BHK cells (Panel 2), BHK cells alone (Panel 3) and U87.CD4-CXCR4 cells (panel 4) were infected with DU151 env VRP at a multiplicity of infection of 3 i.u. per cell. At 18 hours post infection, the cells were examined using light microscopy for the presence of syncytia. The U87.CD4-CCR5 in panel 1 and 2 show clear syncytia, which was absent in the control cell types in the lower panels. In addition, no syntycia were seen in uninfected control cells or VRP-GFP infected cells (data not shown).

- Figures 9A-C. Antigen-specific CTL response in mice to the HIV-1 Clade C VRP-gag vaccine. Eight BALB/c mice were immunized twice, first at day 0 and again at day 28 with 10^3 i.u. (Panel A) or 10^5 i.u. (panels B and C) VRP-gag. Eight days (Panels A and B) or 49 days (Panel C) post-boost, spleen cells were isolated and stimulated *in vitro* with vaccinia virus expressing HIV Gag for 1 week. Chromium release assays were performed using vaccinia-Gag infected target cells (diamond symbols) or control vaccinia alone-infected sc11 target cells (square symbols). Clear HIV Gag-specific lysis was detected in animals vaccinated with the VRP-gag vaccine.

- Figure 10. Diagrammatic representation of the HIV-1 genome. Black bars indicate relative regions of the genome sequenced to generate phylogenetic sequence comparative data for Clade C *gag*, *pol* and *env* gene isolates.

- Figure 11. Phylogenetic comparison of DU422 Clade C Gag isolate with referenced Clade C strains. Consensus clade A, B, D, Mal and SA strains are also shown. DU422 the vaccine strain had 95% amino acid sequence homology to the South African consensus Clade C sequence.

Figure 12. Phylogenetic comparison of DU151 Clade C isolate Env isolate with referenced Clade C strains. DU422 the vaccine strain had 93% amino acid sequence homology to the South African consensus Clade C sequence.

5 Figure 13. Phylogenetic comparison of DU151 Clade C isolate Pol isolate with referenced Clade C strains. DU422 the vaccine strain had 99% amino acid sequence homology to the South African consensus Clade C sequence.

10 Figure 14. DU422 HIV Gag expression as detected by immunofluorescence following electroporation with Gag replicon RNA. BHK cells were electroporated and subjected to immunofluorescence staining with an anti-Gag monoclonal antibody at 24 hours post-electroporation, to demonstrate expression of the Clade C protein.

15 Figure 15. Immunofluorescence detection of DU422 Gag protein expression in BHK cells. BHK cells were infected with VRP-Gag particles and subjected to immunofluorescence staining with an anti-Gag monoclonal antibody at 24 hours post-infection, to demonstrate expression of the Clade C protein.

DETAILED DESCRIPTION OF THE INVENTION

20

As used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" can mean a single pharmaceutical carrier or mixtures of two or more such carriers.

25

The present invention is based on the discovery of a vaccine for the treatment and/or prevention of infection by HIV, comprising novel combinations of isolated nucleic acids, encoding two or more distinct antigens which elicit an immune response in a subject which is effective in treating and/or preventing infection by HIV. In a 30 particular embodiment, the nucleic acids encoding the antigens of the vaccine are

modified to enhance the immunogenicity of the antigen, improve the safety of the vaccine, or both.

- As used herein, the term "isolated nucleic acid" means a nucleic acid separated
5 or substantially free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of nucleic acids can be accomplished by well known techniques such as cell lysis or disruption of virus particles, followed by phenol plus chloroform extraction,
10 followed by ethanol precipitation of the nucleic acids (Sambrook *et al.*, latest edition). The nucleic acids of this invention can be isolated according to methods well known in the art for isolating nucleic acids. Alternatively, the nucleic acids of the present invention can be synthesized according to standard protocols well described in the literature for synthesizing nucleic acids.

15

HIV-VRP Vaccines

- The antigens of this invention can be gene products which are complete proteins or any fragment of a protein determined to be immunogenic by methods well known in
20 the art. Modifications are made to the antigens of this invention to enhance immunogenicity and/or improve the safety of administration of a vaccine containing the antigen. Examples of such modifications are described in the Examples section herein. Furthermore, it is understood that, where desired, other modifications and changes (e.g., substitutions, deletions, additions) may be made in the amino acid sequence of the
25 antigen of the present invention, which may not specifically impart enhanced immunogenicity or improved safety, yet still result in a protein or fragment which retains all of the functional characteristics by which the protein or fragment is defined. Such changes may occur in natural isolates, may be introduced by synthesis of the protein or fragment, or may be introduced into the amino acid sequence of the protein
30 or fragment using site-specific mutagenesis of nucleic acid encoding the protein or

fragment, the procedures for which, such as mis-match polymerase chain reaction (PCR), are well known in the art.

The nucleic acids of this invention can be present in a vector and the vector of
5 this invention can be present in a cell. The vectors and cells of this invention can be in
a composition comprising the cell or vector and a pharmaceutically acceptable carrier.

The vector of this invention can be an expression vector which contains all of
the genetic components required for expression of the nucleic acids of this invention in
10 cells into which the vector has been introduced, as are well known in the art. For
example, the expression vector of this invention can be a vector comprising the helper
RNAs of this invention. Such an expression vector can be a commercial expression
vector or it can be constructed in the laboratory according to standard molecular
biology protocols. The expression vector can comprise viral nucleic acid including, but
15 not limited to, alphavirus, flavivirus, adenovirus, retrovirus and/or adeno-associated
virus nucleic acid. The nucleic acid or vector of this invention can also be in a
liposome or a delivery vehicle which can be taken up by a cell via receptor-mediated or
other type of endocytosis.

20 In another embodiment, the nucleic acids of this invention can be present in a
composition comprising a population of alphavirus replicon particles which comprise
two or more distinct isolated nucleic acids of this invention and wherein the nucleic
acids are each contained within a separate alphavirus replicon particle (herein referred
to as a "VRP"). Thus, the expression vector of the present invention can be an
25 alphavirus replicon particle comprising a nucleic acid encoding an antigen of this
invention.

In a particular embodiment, the present invention provides a composition
comprising two or more isolated nucleic acids selected from the group consisting of an
30 isolated nucleic acid encoding an *env* gene product or an immunogenic fragment

- thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of particles, e.g., virus-like particles, containing the *gag* gene product or the
5 immunogenic fragment thereof, and their release from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity.
- 10 In a preferred embodiment, the invention provides alphavirus replicon particles (VRPs) that can be administered as an HIV vaccine. These HIV-VRPs are propagation defective, single cycle vectors that contain a self-amplifying RNA (replicon RNA), e.g., from VEE, in which the structural protein genes of the virus are replaced by a HIV-1 Clade C *gag* gene or any other HIV antigen to be expressed. Following introduction
15 into packaging (or helper) cells *in vitro*, the replicon RNA is packaged into VRPs by supplying the viral structural proteins in *trans* (helper RNAs).

The present invention further provides a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group
20 consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of particles, such as virus-like
25 particles, containing the *gag* gene product or the immunogenic fragment thereof, from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon
30 particle.

It is also contemplated that the compositions of this invention comprise alphavirus replicon particles in which either the replicon RNA or at least one structural protein comprises one or more attenuating mutations. Thus, the present invention additionally provides a population of alphavirus replicon particles comprising two or 5 more distinct types of such particles selected from the group consisting of 1) particles expressing a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) particles expressing a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment 10 thereof is modified to inhibit release of particles, such as virus-like particles, containing the *gag* gene product or the immunogenic fragment thereof, from a cell, and 3) particles expressing a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment 15 thereof is modified to inhibit reverse transcriptase activity; and wherein the nucleic acids are each contained within a separate alphavirus replicon particle and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

In a preferred embodiment, the population of alphavirus replicon particles 20 comprises particles expressing the nucleic acids encoding *pol*, *env*, and *gag* gene products. In this embodiment, vigorous antigen-specific cellular (e.g., CTL, NK cell and T-helper) and/or humoral (e.g., antibody) responses can be obtained when such particle populations are administered to a subject.

25 In the compositions described above, the *gag* gene product or immunogenic fragment thereof can be modified by mutation of the second codon, whereby a glycine is changed to an alanine. Alternatively, the *gag* gene product or immunogenic fragment thereof can be modified by any other means known in the art for inhibiting the release of particles containing the *gag* gene product or immunogenic fragment 30 thereof from a cell.

Furthermore, in the compositions of this invention, the *pol* gene product or immunogenic fragment thereof can be modified by mutation of the nucleotide sequence encoding the active site motif, whereby YMDD is changed to YMAA or HMAA (the latter providing a convenient site for cloning, see SEQ ID NO:16). The *pol* gene product or immunogenic fragment thereof can also be modified by any means known in the art for inhibiting reverse transcriptase activity.

The *pol* gene product or immunogenic fragment thereof of this invention may be further modified such that the coding sequences for integrase and RNase H are removed, inactivated and/or modified, e.g., by producing only the p51 region of the *pol* gene product. This modification has been shown in some studies to reduce the possibility of formation of replication competent alphavirus particles during production of alphavirus replicon particles comprising the *pol* gene product or immunogenic fragment thereof. This modification can be of the nucleic acid encoding the *pol* gene product or immunogenic fragment thereof according to methods known in the art. Thus, the particles and compositions of this invention can comprise nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof.

In the compositions of this invention, the *gag*, *env* or *pol* gene products or immunogenic fragments thereof can be from any HIV isolate or consensus sequence derived from HIV primary isolates now known or later identified, the isolation and characterization of which are well known in the art. Also, in the compositions of this invention, the *gag*, *env* or *pol* gene products or immunogenic fragments thereof can be produced from the same HIV isolate or HIV consensus sequence or from any combination of HIV isolates or HIV consensus sequences. In the Examples provided herein, the nucleic acid sequences encoding the *env*, *gag* and *pol* gene products of this

invention were selected based on a consensus sequence generated from primary isolates obtained from recent seroconvertors in Kwazulu/Natal in South Africa. Sequence analysis of these isolates identified them as subtype (or clade) C, and in preferred embodiments of the invention, the *env*, *gag* and *pol* genes are from Clade C isolates of
5 HIV.

In preferred embodiments, each of the three HIV genes are derived from one or more of the South African isolates obtained from recent seroconverters in Kwazulu/Natal as described herein (see Figures 11-13 for isolate names). In a further
10 embodiment, the *gag* gene or gene fragment is from a *gag* sequence having 95% or greater amino acid identity with the South African consensus sequence for the *gag* gene. In a specific embodiment, the *gag* gene or fragment thereof is derived from HIV Subtype Clade C isolate DU422 and the *env* and *pol* genes or fragments thereof are derived from HIV isolate DU151.
15

The term "alphavirus" has its conventional meaning in the art and includes the various species of the alphavirus genus, such as Eastern Equine Encephalitis virus (EEE), Venezuelan Equine Encephalitis virus (VEE), Western Equine Encephalitis virus (WEE), Everglades virus, Mucambo virus, Pixuna virus, Sindbis virus, Semliki
20 Forest virus, South African Arbovirus No. 86, Middleburg virus, Chikungunya virus, O=nyong-nyong virus, Ross River virus, Barmah Forest virus, Getah virus, Sagiymama virus, Bebaru virus, Mayaro virus, Una virus, Aura virus, Whataroa virus, Babanki virus, Kyzylagach virus, Highlands J virus, Fort Morgan virus, Ndumu virus, Buggy Creek virus, as well as any specific strains of these alphaviruses (e.g., TR339;
25 Girdwood) and any other virus classified by the International Committee on Taxonomy of Viruses (ICTV) as an alphavirus.

An "alphavirus replicon particle" as used herein is an infectious, replication defective, alphavirus particle which comprises alphavirus structural proteins and further
30 comprises a replicon RNA. The replicon RNA comprises nucleic acid encoding the

alphavirus packaging segment, nucleic acid encoding alphavirus non-structural proteins and a heterologous nucleic acid sequence encoding an antigen of this invention. The non-structural proteins encoded by the replicon RNA may be such proteins as are required for replication and transcription. In a specific embodiment of this invention,

5 the structure of the replicon RNA, starting at the 5' end, comprises the 5' untranslated region of the alphavirus RNA, the non-structural proteins (e.g., nsPs1-4) of the alphavirus, the 26S promoter (also known as the "subgenomic promoter"), the heterologous nucleic acid encoding an HIV antigen, and the 3' untranslated region of the alphavirus RNA. An example of a nucleic acid encoding alphavirus nonstructural

10 proteins that can be incorporated into the embodiments of this invention is SEQ ID NO:2, which encodes the amino acid sequence of SEQ ID NO:3.

Although the alphavirus replicon RNA can comprise nucleic acid encoding one or two alphavirus structural proteins, the replicon RNA does not contain nucleic acid

15 encoding all of the alphavirus structural proteins. The replicon RNA can lack nucleic acid encoding any alphavirus structural protein(s). Thus, the resulting alphavirus replicon particles of this invention are replication defective inasmuch as the replicon RNA does not encode all of the structural proteins required for encapsidation of the replicon RNA and assembly of an infectious virion.

20

As used herein, "alphavirus structural protein" or "structural protein" means the alphavirus proteins required for encapsidation of alphavirus replicon RNA and packaging of the encapsidated RNA into a virus particle. The alphavirus structural proteins include PE2, E2, E3, 6K and E1.

25

The alphavirus replicon particles of this invention can comprise replicon RNA from any of the alphaviruses of this invention. Furthermore, the alphavirus replicon particles of this invention can comprise alphavirus structural proteins from any of the alphaviruses of this invention. Thus, the replicon particles can be made up of replicon

30 RNA and structural proteins from the same alphavirus or from different alphaviruses,

the latter of which would be chimeric alphavirus replicon particles (e.g., a particle comprising Sindbis virus replicon RNA and VEE structural proteins).

The alphavirus replicon particles of this invention can be made by employing a helper cell for expressing an infectious, replication defective, alphavirus particle in an alphavirus-permissive cell. The helper cell includes (a) a first helper RNA encoding (i) at least one alphavirus structural protein, and (ii) not encoding at least one alphavirus structural protein; and (b) a second helper RNA separate from the first helper RNA, the second helper RNA (i) not encoding the at least one alphavirus structural protein encoded by the first helper RNA, and (ii) encoding at least one alphavirus structural protein not encoded by the first helper RNA, such that all of the alphavirus structural proteins assemble together into alphavirus particles in the cell.

The alphavirus structural protein genes can be present on the helper RNAs of this invention in any combination. For example, the helper RNA of this invention can encode the alphavirus capsid and E1, capsid and E2, E1 and E2, capsid only, E1 only, E2 only, etc. It is also contemplated that the alphavirus structural proteins are provided in *trans* from genes located on three separate RNA molecules within the helper cell.

In a preferred embodiment, the helper cell also includes a replicon RNA, which encodes the alphavirus packaging segment and an inserted heterologous RNA. In the embodiment wherein the helper cell also includes a replicon RNA, the alphavirus packaging segment may be, and preferably is, deleted from both the first helper RNA and the second helper RNA. For example, in an embodiment wherein the helper cell includes a replicon RNA encoding the alphavirus packaging segment and an inserted heterologous RNA, the first helper RNA encodes the alphavirus E1 glycoprotein and the alphavirus E2 glycoprotein, and the second helper RNA encodes the alphavirus capsid protein. In a preferred embodiment, the first helper RNA encodes the E3-E2-6k-E1 cassette from an alphavirus. In an alternative embodiment, the cassette encoded on the first helper RNA is referred to as the E3-E2-E1 cassette. A specific embodiment of this aspect of the invention is diagrammed in Figure 3, and an exemplary nucleotide

sequence is SEQ ID NO:11. The replicon RNA, first helper RNA, and second helper RNA are all on separate molecules and are cotransfected, e.g., by electroporation, into the helper cell, which can be any alphavirus permissive cell, as is well known in the art.

5 In an alternative embodiment, the helper cell includes a replicon RNA encoding the alphavirus packaging segment and an inserted heterologous RNA and also includes the alphavirus capsid protein otherwise encoded by the second helper RNA. The first helper RNA encodes the alphavirus E1 glycoprotein and the alphavirus E2 glycoprotein. Thus, the replicon RNA and the first helper RNA are on separate
10 molecules, and the replicon RNA and the second helper RNA are on a single molecule.

The RNA encoding the structural proteins, i.e., the first helper RNA and the second helper RNA, can include one or more attenuating mutations. In a preferred
15 embodiment, either one or both of the first helper RNA and the second helper RNA include at least one attenuating mutation. The attenuating mutations provide the advantage that in the event of RNA recombination within the cell, the coming together of the structural and non-structural genes will produce a virus of decreased virulence.

20 The alphavirus replicon particles of this invention can be made by a) transfecting a helper cell as given above with a replication defective replicon RNA, b) producing the alphavirus particles in the transfected cell, and c) collecting the alphavirus particles from the cell. The replicon RNA encodes the alphavirus packaging segment and a heterologous RNA. The transfected helper cell further includes the first
25 helper RNA and second helper RNA as described above.

As described hereinabove, the structural proteins used to assemble the alphavirus replicon particles of this invention are distributed among one or more helper RNAs (i.e., a first helper RNA and a second helper RNA). As noted herein, one or
30 more structural protein genes may be located on the replicon RNA, provided that at

- least one structural protein gene is deleted from the replicon RNA such that the replicon RNA and resulting alphavirus particle are replication defective. As used herein, the terms "deleted" or "deletion" mean either total deletion of the specified nucleic acid or the deletion of a sufficient portion of the specified nucleic acid to render the nucleic
- 5 acid and/or its resultant gene product inoperative or nonfunctional, in accordance with standard usage. (See, e.g., U.S. Pat. No. 4,650,764 to Temin *et al.*) The term "replication defective" as used herein means that the replicon RNA cannot replicate in the host cell (i.e., produce infectious viral particles) in the absence of the helper RNA. The replicon RNA is replication defective inasmuch as the replicon RNA does not
- 10 include all of the alphavirus structural protein genes required for replication, at least one of the required structural protein genes being deleted therefrom.

In one embodiment, the packaging segment or "encapsidation sequence" is deleted from at least the first helper RNA. In a preferred embodiment, the packaging segment is deleted from both the first helper RNA and the second helper RNA. In a specific embodiment, the second helper RNA is constructed from a VEE cDNA clone, deleting all non-structural proteins (i.e., nsPs1-4), the packaging signal, and the glycoprotein cassette (E3-E2-E1). An example of a plasmid encoding such a second helper RNA is provided in Figure 2, and an exemplary nucleotide sequence for such a 20 second helper RNA is SEQ ID NO:8.

In the preferred embodiment wherein the packaging segment is deleted from both the first helper RNA and the second helper RNA, preferably the helper cell contains a replicon RNA in addition to the first helper RNA and the second helper 25 RNA. The replicon RNA encodes the packaging segment and an inserted heterologous RNA encoding an HIV antigen or a fragment thereof. Typically, the inserted heterologous RNA encodes a gene product which is expressed by the target cell, and includes the promoter and regulatory segments necessary for the expression of that gene product in that cell.

- In another preferred embodiment, the replicon RNA, the first helper RNA and the second helper RNA are provided on separate molecules such that a first molecule, i.e., the replicon RNA, encodes the packaging segment and the inserted heterologous RNA, a second molecule, i.e., the first helper RNA, encodes at least one but not all of the required alphavirus structural proteins, and a third molecule, i.e., the second helper RNA, encodes at least one but not all of the required alphavirus structural proteins. For example, in one preferred embodiment of the present invention, the helper cell includes a set of RNAs which include (a) a replicon RNA encoding an alphavirus packaging sequence and an inserted heterologous RNA, (b) a first helper RNA encoding the alphavirus E1 glycoprotein and the alphavirus E2 glycoprotein, and (c) a second helper RNA encoding the alphavirus capsid protein, so that the alphavirus E1 glycoprotein, the alphavirus E2 glycoprotein and the capsid protein assemble together into alphavirus particles containing the replicon RNA in the helper cell.
- In an alternate embodiment, the replicon RNA and the first helper RNA are on separate molecules, and the replicon RNA and the second helper RNA are on a single molecule together, thereby providing a first molecule, i.e., the first helper RNA, encoding at least one but not all of the required alphavirus structural proteins, and a second molecule, i.e., the replicon RNA and second helper RNA, encoding the packaging segment, the inserted heterologous gene product and the structural protein(s) not encoded by the first helper. Thus, one or more structural protein(s) is encoded by the second helper RNA, but the second helper RNA is located on the second molecule together with the replicon RNA. For example, in one preferred embodiment of the present invention, the helper cell includes a set of RNAs including (a) a replicon RNA encoding an alphavirus packaging sequence, an inserted heterologous RNA, and an alphavirus capsid protein, and (b) a first helper RNA encoding the alphavirus E1 glycoprotein and the alphavirus E2 glycoprotein so that the alphavirus E1 glycoprotein, the alphavirus E2 glycoprotein and the capsid protein assemble together into alphavirus particles in the helper cell.

The present invention also contemplates alphavirus replicon particles which comprise replicon RNA encoding more than one heterologous gene product. For expression of more than one heterologous nucleic acid from a single replicon RNA, a promoter can be inserted upstream of each heterologous nucleic acid on the replicon 5 RNA, such that the promoter regulates expression of the heterologous nucleic acid, resulting in the production of more than one antigen from a single replicon RNA. Another embodiment contemplates the insertion of an IRES sequence, such as the one from the picornavirus, EMC virus, between the heterologous genes downstream from a 26S promoter of the replicon, thus leading to translation of multiple antigens from a 10 single replicon.

In one preferred embodiment of the present invention, the RNA encoding the alphavirus structural proteins, i.e., the capsid, E1 glycoprotein and/or E2 glycoprotein, 15 contains at least one attenuating mutation. It is further contemplated that the RNA encoding the non-structural proteins can contain at least one attenuating mutation. The phrases "attenuating mutation" and "attenuating amino acid," as used herein, mean a nucleotide mutation or an amino acid coded for in view of such a mutation which result in a decreased probability of causing disease in its host (i.e., a loss of virulence), in 20 accordance with standard terminology in the art, See, e.g., Davis *et al.* (1980). The mutation can be, for example, a substitution mutation or an in-frame deletion mutation. The phrase "attenuating mutation" excludes mutations which would be lethal to the virus. Thus, according to this embodiment, the E1 RNA and/or the E2 RNA and/or the capsid RNA can include at least one attenuating mutation. In a more preferred 25 embodiment, the E1 RNA and/or the E2 RNA and/or the capsid RNA includes at least two, or multiple, attenuating mutations. The multiple attenuating mutations may be positioned in either the first helper RNA or in the second helper RNA, or they may be distributed randomly with one or more attenuating mutations being positioned in the first helper RNA and one or more attenuating mutations positioned in the second helper 30 RNA. Appropriate attenuating mutations will be dependent upon the alphavirus used,

as is well known in the art.

For example, when the alphavirus is VEE, suitable attenuating mutations can be in codons at E2 amino acid position 76 which specify an attenuating amino acid,
5 preferably lysine, arginine, or histidine as E2 amino acid 76; codons at E2 amino acid position 120 which specify an attenuating amino acid, preferably lysine as E2 amino acid 120; codons at E2 amino acid position 209 which specify an attenuating amino acid, preferably lysine, arginine, or histidine as E2 amino acid 209; codons at E1 amino acid 272 which specify an attenuating mutation, preferably threonine or serine as E1
10 amino acid 272; codons at E1 amino acid 81 which specify an attenuating mutation, preferably isoleucine or leucine as E1 amino acid 81; and codons at E1 amino acid 253 which specify an attenuating mutation, preferably serine or threonine as E1 amino acid 253; and the combination mutation of the deletion of E3 codons 56-59 together with codons at E1 amino acid 253 which specify an attenuating mutation, as provided
15 herein. Other suitable attenuating mutations within the VEE genome will be known to those skilled in the art.

In an alternate embodiment, wherein the alphavirus is the South African Arbovirus No. 86 (S.A.A.R.86), suitable attenuating mutations can be, for example, in codons at nsP1 amino acid position 538 which specify an attenuating amino acid, preferably isoleucine as nsP1 amino acid 538; codons at E2 amino acid position 304 which specify an attenuating amino acid, preferably threonine as E2 amino acid 304; codons at E2 amino acid position 314 which specify an attenuating amino acid, preferably lysine as E2 amino acid 314; codons at E2 amino acid position 376 which
25 specify an attenuating amino acid, preferably alanine as E2 amino acid 376; codons at E2 amino acid position 372 which specify an attenuating amino acid, preferably leucine as E2 amino acid 372; codons at nsP2 amino acid position 96 which specify an attenuating amino acid, preferably glycine as nsP2 amino acid 96; codons at nsP2 amino acid position 372 which specify an attenuating amino acid, preferably valine as
30 nsP2 amino acid 372; in combination, codons at E2 amino acid residues 304, 314, 372

and 376; codons at E2 amino acid position 378 which specify an attenuating amino acid, preferably leucine as E2 amino acid 378; codons at nsP2 amino acid residue 372 which specify an attenuating mutation, preferably valine as nsP2 amino acid 372; in combination, codons at nsP2 amino acid residues 96 and 372 attenuating substitution 5 mutations at nsP2 amino acid residues 96 and 372; codons at nsP2 amino acid residue 529 which specify an attenuating mutation, preferably leucine, at nsP2 amino acid residue 529; codons at nsP2 amino acid residue 571 which specify an attenuating mutation, preferably asparagine, at nsP2 amino acid residue 571; codons at nsP2 amino acid residue 682 which specify an attenuating mutation, preferably arginine, at nsP2 10 amino acid residue 682; codons at nsP2 amino acid residue 804 which specify an attenuating mutation, preferably arginine, at nsP2 amino acid residue 804; codons at nsP3 amino acid residue 22 which specify an attenuating mutation, preferably arginine, at nsP3 amino acid residue 22; and in combination, codons at nsP2 amino acid residues 529, 571, 682 and 804, and at nsP3 amino acid residue 22, specifying attenuating 15 amino acids at nsP2 amino acid residues 529, 571, 682 and 804 and at nsP3 amino acid residue 22. Other suitable attenuating mutations within the S.A.A.R.86 genome will be known to those skilled in the art.

The alphavirus capsid gene used to make alphavirus replicon particles can also 20 be subjected to site-directed mutagenesis. The altered capsid protein provides additional assurance that recombination to produce the virulent virus will not occur. The altered capsid protein gene which functions in particle assembly but not in autoproteolysis provides helper function for production of replicon particles, but does not allow for production of a viable recombinant. The capsid residues required for 25 proteolytic function are known (Strauss *et al.*, 1990).

Suitable attenuating mutations useful in embodiments wherein any of the alphaviruses of this invention are employed are known to or can be identified by those skilled in the art using routine protocols. Attenuating mutations may be introduced into 30 the RNA by performing site-directed mutagenesis on the cDNA which encodes the

RNA, in accordance with known procedures. See Kunkel (1985), the disclosure of which is incorporated herein by reference in its entirety. Alternatively, mutations may be introduced into the RNA by replacement of homologous restriction fragments in the cDNA which encodes for the RNA, in accordance with known procedures. The 5 identification of a particular mutation in an alphavirus as attenuating is done using routine experimentation according to methods well known in the art.

Preferably, the helper RNA of this invention includes a promoter. It is also preferred that the replicon RNA includes a promoter. Suitable promoters for inclusion 10 in the helper RNA and replicon RNA are well known in the art. One preferred promoter is the alphavirus 26S promoter, although many suitable promoters are available, as is well known in the art.

In the system wherein a first helper RNA, a second helper RNA, and a replicon 15 RNA are all on separate molecules, if the same promoter is used for all three RNAs, then a homologous sequence between the three molecules is provided. Thus, it is advantageous to employ different promoters on the first and second helper RNAs to provide further impediment to RNA recombination that might produce virulent virus. It is preferred that the selected promoter is operative with the non-structural proteins 20 encoded by the replicon RNA molecule.

The infectious, replication defective, alphavirus particles of this invention are prepared according to the methods disclosed herein in combination with techniques known to those skilled in the art. The methods include, for example, transfecting an 25 alphavirus-permissive cell with a replication defective replicon RNA including the alphavirus packaging segment and an inserted heterologous RNA, a first helper RNA encoding at least one alphavirus structural protein, and a second helper RNA encoding at least one alphavirus structural protein which is different from that encoded by the first helper RNA; producing the alphavirus particles in the transfected cell; and 30 collecting the alphavirus particles from the cell.

- Methods for transfecting the alphavirus-permissive cell with the replicon RNA and helper RNAs can be achieved, for example, by (i) treating the cells with DEAE-dextran, (ii) by lipofection, by treating the cells with, for example, LIPOFECTIN, and (iii) by electroporation, with electroporation being a preferred
- 5 means of achieving RNA uptake into the alphavirus-permissive cells. Examples of these techniques are well known in the art, see e.g., U.S. Pat. No. 5,185,440 to Davis *et al.*, and PCT Publication No. WO 92/10578 to Biooption AB, the disclosures of which are incorporated herein by reference in their entirety.
- 10 The steps of producing the infectious viral particles in the cells may also be carried out using conventional techniques. See e.g., U.S. Patent No. 5,185,440 to Davis *et al.*, PCT Publication No. WO 92/10578 to Biooption AB, and U.S. Patent No. 4,650,764 to Temin *et al.* (although Temin *et al.*, relates to retroviruses rather than alphaviruses). The infectious viral particles may be produced by standard cell culture
- 15 growth techniques.

The steps of collecting the infectious alphavirus particles may also be carried out using conventional techniques. For example, the infectious particles may be collected by cell lysis, or collection of the supernatant of the cell culture, as is known in

20 the art. See e.g., U.S. Patent No. 5,185,440 to Davis *et al.*, PCT Publication No. WO 92/10578 to Biooption AB, and U.S. Patent No. 4,650,764 to Temin *et al.* (although Temin *et al.* relates to retroviruses rather than alphaviruses). Other suitable techniques will be known to those skilled in the art. Optionally, the collected infectious alphavirus particles may be purified, if desired. Purification techniques for viruses are well known

25 to those skilled in the art, and these are suitable for the purification of small batches of infectious alphavirus particles.

Thus, the present invention provides a method of making the populations of alphavirus replicon particles of this invention comprising:

30 A) (a) providing a first helper cell for producing a first population of infectious,

defective alphavirus particles, comprising in an alphavirus-permissive cell:

(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

5 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein;

10 and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

15 and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains
20 no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
(c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of
25 infectious, defective alphavirus particles, comprising in an alphavirus-permissive cell:

(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles

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containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) providing a third helper cell for producing a third population of infectious, defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity or is modified to inactivate or delete integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment

thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein

not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and
unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

(b) producing the alphavirus particles in the helper cell; and

(c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the populations of alphavirus replicon particles.

In a preferred embodiment, as noted above, the method provided also includes a mutation in the *pol* gene product or immunogenic fragment thereof resulting in inactivation or deletion of integrase and RNase H functions of the *pol* gene product or immunogenic fragment thereof. In a specific embodiment of this method, the region of the *pol* gene encoding the RNase H and integrase function of the *pol* gene product or immunogenic fragment thereof has been deleted.

A method of making the populations of alphavirus replicon particles of this invention, wherein the particles comprise at least one attenuating mutation, is also provided, comprising:

- A) (a) providing a first helper cell for producing a first population of infectious, defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;
- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells;
- B) providing a second helper cell for producing a second population of infectious,

defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit release of particles, such as virus-like particles, containing the *gag* gene product or the immunogenic fragment thereof from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - 5 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - 10 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
 - 15 and with at least one of said helper RNAs lacking an alphavirus packaging signal;
 - 20 wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said
 - 25 first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;
 - (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells;
- C) providing a third helper cell for producing a third population of infectious,
- 30 defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity or is modified to inactivate or delete integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- 5 (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- 10 (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- 15 and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- 20 wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said
- 25 first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;
- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and
- D) combining the first population of alphavirus particles produced from the first
- 30 helper cell, the second population of alphavirus particles produced from the second

helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the populations of alphavirus replicon particles of the present invention comprising at least one attenuating mutation.

- 5 In a preferred embodiment, as noted above, the method provided above can include a further mutation in the *pol* gene product or immunogenic fragment thereof resulting in inactivation or deletion of integrase and RNase H functions of the *pol* gene product or immunogenic fragment thereof. In a specific embodiment of this method, the region of the *pol* gene encoding the RNase H and integrase function of the *pol* gene
10 product or immunogenic fragment thereof has been deleted.

It is also contemplated regarding the method described above, that not all of the first, second and third populations of alphavirus particles do not all have to comprise an attenuating mutation. For example, the first population may comprise attenuating
15 mutations, but the second and third populations may not, etc.

The present invention further provides the compositions of the present invention which are produced by the methods of this invention.

- 20 The compositions and methods of this invention which incorporate attenuating mutations into the alphavirus replicon particles forming the composition and/or produced by the methods include purified compositions and methods of purification based on the presence of the attenuating mutations. In particular, certain attenuating mutations in the alphavirus structural proteins introduce heparin binding sites into these
25 proteins which are present on the surface of the alphavirus replicon particles. As an example, the V3014 E2 glycoprotein (SEQ ID NO:12 and SEQ ID NO:13) has a mutation in which a lysine is substituted for the glutamic acid at amino acid position 209. This mutation, which creates a more positively charged glycoprotein, increases the affinity of this protein for heparin. Thus, it is possible to purify such particles using
30 heparin affinity chromatography. Such chromatography can be performed using any of

several commercially available resins to which heparin has been bound. The source of heparin is variable; the commercially available resins currently use porcine heparin. The choice of resin will be based on its relative ease of use in a scaled-up, GMP-compliant process, e.g., price, column packing limitations, and potential for easy
5 sanitization. The use of heparin affinity chromatography results in a substantial purification of the VRPs with very little loss of material, and it is a scalable purification step. In a preferred embodiment, a heparin affinity chromatography step results in between an 8- to 27-fold reduction in total protein per ml, or from a 300- to 1000-fold reduction in total protein per VRP. Thus, the present invention provides heparin
10 affinity-purified alphavirus replicon particles containing attenuating mutations which are useful as clinical trial material and commercial product. The present invention also provides methods for preparing purified alphavirus replicon particles containing attenuating mutations comprising the use of heparin affinity chromatography, as described in the Examples provided herein. These particles can also be present in a
15 composition of this invention.

The alphavirus replicon particles of this invention can also be made in a cell free system. Such replicon particles are herein referred to as virosomes. In a specific embodiment of the method, such particles are constructed from a mixture containing
20 replicon RNA that does not encode all of the alphavirus structural proteins, purified glycoproteins E1 and E2, one or more non-cationic lipids, such as lecithin, and detergent. Detergent is slowly removed from the mixture to allow formation of lipid bilayers with incorporated RNA and glycoproteins.

25 In preferred embodiments of the methods of this invention, the glycoproteins E1 and E2 could be expressed in any recombinant protein expression system capable of glycosylation of mammalian proteins, such as stably transformed cell lines, for example CHO cells, or viral vector expression systems such as vaccinia, baculovirus, herpes virus, alphavirus or adenovirus. In a preferred embodiment, following expression of
30 the proteins, the E1 and E2 glycoproteins are purified from contaminating cellular

- proteins in the expression supernatant. The purification of these glycoproteins can be achieved by affinity chromatographic column purification, for example using lectin-, heparin-, or antibody-affinity columns. This affinity purification step may be preceded by selective precipitation or selective extraction from the expression system supernatant
- 5 by methods including, but not limited to, ammonium sulfate precipitation or detergent extraction respectively. Final polishing steps of purification may include ion-exchange chromatography or buffer exchange, for example, and tangential flow methods to generate purified glycoproteins suitable for virosome assembly.
- 10 Thus, the present invention provides a method of producing alphavirus replicon virosomes, comprising: a) combining alphavirus replicon RNA, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and b) gradually removing detergent, whereby alphavirus replicon virosomes are produced. This method is described in more detail in the Examples section herein.
- 15 The present invention also provides alphavirus replicon virosomes comprising an alphavirus replicon RNA encapsidated by a lipid bilayer in which alphavirus glycoproteins are embedded. The replicon RNA can be from any alphavirus and the glycoproteins can be from any alphavirus. In a specific embodiment, the alphavirus
- 20 glycoproteins are VEE E1 and E2. The advantage of the alphavirus replicon virosomes is the ease of preparation, their stability, and their purity, since they are devoid of any cellular components being made in a cell free system.
- The helper cells, RNAs and methods of the present invention are useful in *in*
- 25 *vitro* expression systems, wherein the inserted heterologous RNA located on the replicon RNA encodes a protein or peptide which is desirably produced *in vitro*. The helper cells, RNAs, methods, compositions and pharmaceutical formulations of the present invention are additionally useful in a method of administering a protein or peptide to a subject in need of the desired protein or peptide, as a method of treatment
- 30 or otherwise.

It is contemplated that the nucleic acids, vectors and alphavirus replicon particles of this invention can be administered to a subject to impart a therapeutic or beneficial effect. Therefore, the nucleic acids, vectors and particles of this invention can be present in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector of this invention, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art (see, e.g., *Remington's Pharmaceutical Science*; latest edition).

Pharmaceutical formulations of this invention, such as vaccines, of the present invention can comprise an immunogenic amount of the alphavirus replicon particles as disclosed herein in combination with a pharmaceutically acceptable carrier. An "immunogenic amount" is an amount of the infectious alphavirus particles which is sufficient to evoke an immune response (humoral and/or cellular immune response) in the subject to which the pharmaceutical formulation is administered. An amount of from about 10^3 to about 10^7 replicon-containing particles, and preferably, about 10^4 to about 10^6 replicon-containing particles per dose is believed suitable, depending upon the age and species of the subject being treated. Exemplary pharmaceutically acceptable carriers include, but are not limited to, sterile pyrogen-free water and sterile pyrogen-free physiological saline solution.

25

Subjects which may be administered immunogenic amounts of the infectious, replication defective alphavirus particles of the present invention include, but are not limited to, human and animal (e.g., horse, donkey, mouse, hamster, monkey) subjects. Administration may be by any suitable means, such as intraperitoneal or intramuscular injection.

Pharmaceutical formulations for the present invention can include those suitable for parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous and intraarticular) administration. Alternatively, pharmaceutical formulations of the present invention may be suitable for administration to the mucous membranes of a subject 5 (e.g., intranasal administration). The formulations may be conveniently prepared in unit dosage form and may be prepared by any of the methods well known in the art.

Thus, the present invention provides a method for delivering nucleic acids and vectors (e.g., alphavirus replicon particles; virosomes) encoding the antigens of this 10 invention to a cell, comprising administering the nucleic acids or vectors to a cell under conditions whereby the nucleic acids are expressed, thereby delivering the antigens of this invention to the cell. The nucleic acids can be delivered as naked DNA or in a vector (which can be a viral vector) or other delivery vehicles and can be delivered to cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., 15 uptake of naked DNA, viral infection, liposome fusion, endocytosis and the like). The cell can be any cell which can take up and express exogenous nucleic acids.

Further provided herein is a method of inducing an immune response to an HIV antigen of this invention in a subject, comprising administering to the subject an 20 immunogenic amount of the particles, virosomes and/or composition of this invention, in a pharmaceutically acceptable carrier.

A method of treating and/or preventing infection by HIV in a subject is also provided herein, comprising administering to the subject an effective amount of the 25 particles, virosomes and/or compositions of this invention, in a pharmaceutically acceptable carrier.

The subject of this invention can be any animal in which an immune response can be induced or in which an infection by HIV can be treated and/or prevented. In a 30 preferred embodiment, the subject of this invention is a mammal and most preferably is

a human.

Protocols and data regarding the testing of the compositions of this invention in animals and protocols for administration to humans are provided in the Examples 5 herein.

In a particular embodiment, the present invention provides an isolated nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the integrase, RNase H and reverse transcriptase 10 functions of the *pol* gene product or immunogenic fragment thereof have been inactivated or deleted. Such a modification has been shown in some studies to facilitate inhibition of the formation of replication competent alphavirus particles during production of alphavirus replicon particles comprising the *pol* gene product or immunogenic fragment thereof.

15

Also provided herein is a composition comprising the *pol*-expressing nucleic acid described above, a vector comprising the nucleic acid and a cell comprising the vector. The *pol*-expressing nucleic acid can also be present in an alphavirus replicon particle comprising the nucleic acid.

20

As noted above, the nucleic acid encoding the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in the inhibition of reverse transcriptase activity. In a preferred embodiment, a mutation is introduced at the active site motif that results in inhibition of reverse transcriptase activity. Such a mutation 25 may remove the DNA binding domain of the enzyme, for example. A mutation from YMDD to YMAA or HMAA at this motif is an example of such a mutation.

The present invention additionally provides a method of making an alphavirus replicon particle comprising nucleic acid encoding a *pol* gene product or immunogenic 30 fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or

immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions from the *pol* gene product or immunogenic fragment thereof, comprising

- A) providing a helper cell for producing an infectious, defective alphavirus
5 particle, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to delete or inactivate RNase H, integrase and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper
25 RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;
- (B) producing the alphavirus particles in the helper cell; and
 - 30 (C) collecting the alphavirus particles from the helper cell.

In the method provided above, at least one of the replicon RNA, the first helper RNA, and the one or more additional helper RNA(s) can comprise one or more attenuating mutations, as described herein.

- 5 In a specific embodiment of this method, a mutation is introduced at the active site motif in the *pol* gene product or immunogenic fragment thereof that results in inhibition of reverse transcriptase activity. Such a mutation may remove the DNA binding domain of the enzyme, for example. A mutation from YMDD to YMAA or HMAA at this motif is an example of such a mutation.

10

Also provided herein is an alphavirus replicon particle expressing the *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, produced according to any of the above methods.

- 15 In a further embodiment, the present invention provides a method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of a composition comprising an alphavirus particle comprising nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, in a pharmaceutically acceptable carrier.

- 20 Furthermore, the present invention provides a method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an effective amount of a composition comprising an alphavirus particle comprising nucleic acid encoding a *pol* gene product or immunogenic fragment thereof

of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, in a pharmaceutically acceptable carrier.

5

- In preferred embodiments of the methods of this invention, the subject is administered an effective amount of a population of alphavirus particles comprising particles expressing (1) nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation or deletion of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, (2) nucleic acid encoding a *gag* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit release of *gag* gene product or the immunogenic fragment thereof from a cell, and (3) nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus in a pharmaceutically acceptable carrier.

- In further preferred embodiments, the population of alphavirus particles comprises particles expressing (1) nucleic acid encoding a *gag* gene sequence that has at least 92% identity with SEQ ID NO:4; (2) nucleic acid encoding a *pol* gene sequence that has at least 99% identity with SEQ ID NO:15; and (3) nucleic acid encoding an *env* gene sequence with at least 95% identity with SEQ ID NO:18. In a specific embodiment, the population of alphavirus particles comprises particles expressing (1) nucleic acid of SEQ ID NO:4, (2) nucleic acid of SEQ ID NO:15, and (3) nucleic acid of SEQ ID NO:18.

EXAMPLES

- 30 The following examples are provided to illustrate the present invention, and

- should not be construed as limiting thereof. In these examples, nm means nanometer, mL means milliliter, pfu/mL means plaque forming units/milliliter, VEE means Venezuelan Equine Encephalitis virus, EMC means encephalomyocarditis virus, BHK means baby hamster kidney cells, HA means hemagglutinin gene, N means nucleocapsid, FACS means fluorescence activated cell sorter, and IRES means internal ribosome entry site. The expression "E2 amino acid (e.g., lys, thr, etc.) number" indicates the designated amino acid at the designated residue of the E2 gene, and is also used to refer to amino acids at specific residues in the E1 protein and in the E3 protein, respectively.

10

EXAMPLE 1
VEE Replicon Particles as Vaccines

Replicon particles for use as a vaccine are produced using the VEE-based vector system, originally developed from a full-length, infectious cDNA clone of the RNA genome of VEE (Figure 1 in Davis *et al.*, 1989). In this Example, one or more attenuating mutations (Johnston and Smith, 1988; Davis *et al.*, 1990) have been inserted into the clone to generate attenuated VEE vaccine vectors (Davis *et al.*, 1991; 1995; Grieder *et al.*, 1995).

20

As described herein, these constructs are genetically modified to create an RNA replicon (i.e., an RNA that self-amplifies and expresses), and one or more helper RNAs to allow packaging. The replicon RNA expresses an HIV gene, e.g., the Clade C HIV-1 *gag* gene. The replicon RNA is packaged into virus-like particles (herein referred to as "virus replicon particles" or "VRPs") that are infectious for only one cycle. During this cycle, the characteristics of the alphavirus-based vector result in very high levels of expression of the replicon RNA in cells to which the VRP is targeted, e.g., cells of the lymph node.

30

In the cytoplasm of the target cell, the replicon RNA is first translated to

produce the viral replicase proteins necessary to initiate self-amplification and expression. In this Example, the HIV-1 Clade C *gag* gene is encoded by a subgenomic mRNA, abundantly transcribed from a negative-sense replicon RNA intermediate, leading to high-level expression of the HIV-1 Clade C *gag* gene product. Since the
5 VEE structural protein genes are not encoded by the replicon RNA, progeny virion particles are not assembled, thus limiting the replication to a single cycle within the infected target cell.

Importantly, only the replicon RNA is packaged into VRPs, as the helper RNAs
10 lack the *cis*-acting packaging sequence required for encapsidation. The "split helper" or bipartite system (see Example 4) greatly reduces the chance for an intact genome being assembled by recombination, and as a back-up safety feature, one or more highly attenuating mutations, such as those contained in the glycoprotein genes in V3014 (Grieder *et al.*, 1995), are incorporated.
15

Overall, the design of the VRPs incorporates several layered and redundant safety features. In addition to the above-described split helper system and attenuating mutations, over one-third of the genome of the virus has been removed, creating a defective genome which prevents spread from the initially infected target cell.
20 Nonetheless, if a statistically rare recombination event occurs to yield replication competent virus (RCV), the resulting virus would be a highly attenuated VEE strain.

EXAMPLE 2

Construction of VEE Replicon

The VEE structural protein genes (C-PE2-6K-E1) are removed from a cDNA clone (pV4031) which contained two attenuating mutations (E2 lys 209, E1 thr 272), and a duplication of the 26S subgenomic RNA promoter sequence immediately downstream from the 3'-end of the E1 glycoprotein gene, followed by a multiple
30 cloning site as described in U.S. Pat. No. 5,505,947 to Johnston *et al.* The pV4031

plasmid DNA is digested to completion with ApaI restriction enzyme, which cuts the VEE genomic sequence at nucleotide 7505 (numbered from the 5'-end of the genome sequence). A second recognition site for this enzyme is found in the duplicate 26S subgenomic promoter. Therefore, digestion of pV4031 with ApaI produces two DNA
5 fragments, one containing the VEE nonstructural genes (e.g. SEQ ID NO:2) and a single copy of the 26S subgenomic RNA promoter followed by a multiple cloning site, and a second smaller fragment containing a 26S subgenomic RNA promoter followed by the VEE structural genes. The large fragment is isolated and religated to produce the replicon, pVR2. In this example, as well as in the construction of the helper plasmids
10 (Example 3), a kanamycin resistance gene (SEQ ID NO:6, encoding amino acid sequence as in SEQ ID NO:7) is present in the plasmids to aid in the cloning manipulations.

EXAMPLE 3

15 Construction of Helper Plasmids

The starting materials for the helper plasmids are four full-length cDNA clones: V3000, the virulent Trinidad donkey strain of VEE, three clones with attenuating mutations, pV3014 (E2 lys 209, E1 thr 272), V3519 (E2 lys 76, E2 lys 209, E1 thr 272)
20 and V3526 (deletion of E3 56-59, E1 ser 253), which are in the genetic background of Trinidad donkey strain VEE. Several different helper plasmids have been made by using unique or rare restriction sites in the full-length cDNA clone to delete portions of the nonstructural protein region. The full-length clone is digested with one or two restriction enzymes, the larger DNA fragment is isolated and then religated to form a
25 functional plasmid. *In vitro* RNA transcripts from these plasmids upon transfection of tissue culture cells would not encode a functional RNA replication complex, and also would not include an encapsidation signal. The helper constructs differ in the size of the nonstructural gene deletion. The helper constructs are designated by the attenuated mutant clone used in their construction, and by the percentage of the nonstructural
30 region deleted. The following helper constructs were generated:

V3014Δ520-7507(93%)
V3519Δ520-7507(93%)
V3526Δ520- 7505(93%)
V3014Δ520-6965(87%)
5 V3519Δ1687-7507(78%)
V3014Δ2311-7505(70%)
V3519Δ3958-7507(47%)
V3526Δ520-7505(93%)
V3014Δ3958-7505(47%)
10 V3519Δ1955-3359(19%)
V3014Δ520-3954(46%)
V3014Δ1955-3359(19%)
V3014Δ1951-3359(19%)
V3014Δ2311-3055(10%)
15 V3014Δ2307-3055(10%)

EXAMPLE 4

Construction of Bipartite RNA Helper Plasmids

20 A bipartite helper system is constructed as described herein. The V3014Δ520-7505(93%) helper is used to construct an additional deletion of the E2 and E1 glycoprotein genes by digestion with HpaI restriction enzyme and ligation, resulting in deletion of the sequence between nucleotide 8494 (in the E3 gene) and nucleotide 11,299 (near the 3'-end of the E1 gene). *In vitro* RNA transcripts of this glycoprotein
25 helper plasmid (presented graphically in Figure 2; an exemplary nucleotide sequence for such a plasmid is SEQ ID NO:8, including the nucleotide sequence (SEQ ID NO:9 and the amino acid sequence (SEQ ID NO:10 of the VEE capsid), when electroporated into BHK cells with a replicon RNA, are replicated and transcribed to give a mRNA encoding only the capsid protein of VEE.

The second member of the bipartite helper is constructed from the same original helper plasmid 3014Δ5207505(93%) by cleavage with Tth11I restriction enzyme (at nucleotide 7544) and SpeI restriction enzyme (at nucleotide 8389), resulting in deletion of the capsid gene, followed by insertion of a synthetic double-stranded oligonucleotide 5 with Tth11I and SpeI termini. The inserted sequence restored the downstream portion of the 26S promoter and an ATG initiation codon followed by a Ser codon, such that the first amino acid residue of E3 (Ser) is the first codon following the inserted AUG. The resulting glycoprotein helper plasmid is presented graphically in Figure 3, and an exemplary nucleic acid sequence for such a plasmid is SEQ ID NO:11, encoding the 10 VEE glycoproteins (E3-E2-6kD-E1), SEQ ID NO:12. The *in vitro* transcript of this plasmid, when transfected into a cell with replicon RNA, will produce the VEE glycoproteins (SEQ ID NO:13). Co-electroporation of both of these helper RNAs into a cell with replicon RNA results in production of infectious particles containing only replicon RNA.

15

Other than the 5' and 3' ends and the 26S promoters (40 nucleotides) of these helper RNAs, the only sequence in common between the capsid and glycoprotein helpers is the sequence from 8389 to 8494 (106 nucleotides)

20

EXAMPLE 5

VEE REPLICON PARTICLES EXPRESSING HIV GENES

The vaccines of this invention are exemplified by the use of a propagation defective, replicon particle vector system derived from an attenuated strain of 25 Venezuelan equine encephalitis virus (VEE) to create a mixture of VEE replicon particles individually expressing HIV-1 *gag*, *pol*, or *env* genes. The three genes used in this Example were selected based on homology to consensus sequences generated from primary isolates obtained from recent seroconverters in Kwazulu/Natal. Plasma samples from approximately 20 recent seroconverters in the Durban/Hlabisa cohort and 30 a similar number of HIV-positive, asymptomatic individuals were collected. HIV viral

RNA was isolated from the plasma, and the sequences of the *gag*, *pol* and *env* genes were analyzed. Two regions from each gene were amplified, and the resulting PCR products were sequenced (see Figure 10 for regions analyzed). A consensus sequence was derived for each gene, and the sequences of each isolate were compared to the 5 derived consensus. All isolates were found to be Subtype C of HIV, thus confirming the predominance of this subtype in South Africa.

A. CONSTRUCTION OF THE Gag-VRP VACCINE

10 Described herein is the design and manufacture of VEE replicon particles (VRPs) engineered to express the *gag* gene from a Subtype C isolate of HIV-1. The main purpose of this single antigen vaccine is to establish a safety profile for VRPs in healthy human subjects. Optimally, the HIV-Gag-VRPs will be formulated as a component of a trivalent vaccine, also containing HIV-Pol-VRP and HIV-gp160-VRP
15 (*env*) made in analogous procedures to the one described herein for HIV-Gag-VRPs.

In this Example, the VEE particles are based on the V3014 glycoprotein helper plasmid (Figure 3, SEQ ID NO:12 and SEQ ID NO:13), which harbors two highly attenuating mutations, one in E2 and the other in E1 (Grieder *et al.*, 1995). The V3014 20 glycoprotein helper RNA is able to package VRPs with significantly greater efficiency than the glycoprotein helper RNA derived from V3526 (Pushko *et al.*, 1997). Nonetheless, safety of the VRP vector system has not been compromised since detailed pathogenesis studies clearly have shown V3014 to be avirulent in adult mice by 25 subcutaneous inoculation (Grieder *et al.*, 1995). V3014 was found to be significantly impaired in its ability to reach and spread beyond the draining lymph node following subcutaneous inoculation. Unlike wild-type V3000, V3014 does not establish a viremia and does not reach the brain. In addition, on rare occasions when found, histopathological lesions in the periphery were much less severe than those induced by wild-type V3000 (Grieder *et al.*, 1995). Following inoculation with V3014, adult mice 30 are protected against lethal wild-type VEE infection.

The attenuated phenotype of V3014 also was observed in VEE challenge studies in horses. Animals inoculated subcutaneously with V3014 showed no significant leukopenia or febrile response compared to mock-vaccinated controls. In addition, results indicated that these animals were completely protected against virulent

5 VEE (V3000) challenge.

Taken together, these data indicate that if the rare recombination event did occur during VRP assembly to yield RCV, the worst case scenario would be the generation of a highly attenuated strain of VEE.

10

B. SELECTION AND CLONING OF THE HETEROLOGOUS ANTIGEN

The exemplary HIV genes used in this invention, *gag*, *pol* and *env*, are derived from Subtype C (Clade C) viruses isolated from likely Phase III clinical trial sites in 15 South Africa. The HIV infection rate in South Africa and its long established virology and public health infrastructure make this country an attractive choice for clinical testing of HIV vaccines. Focused sequencing and phylogenetic analysis of the *gag*, *pol*, and *env* genes of these isolates has allowed the selection of genes representative of the Clade C isolates circulating in this region of Africa.

20

1. HIV-1 Clade C *gag* gene

Two 400 bp regions of the *gag* gene were sequenced from approximately 30 plasma samples collected from HIV seropositive individuals in South Africa. A South African consensus sequence was then determined for the *gag* gene as well as a 25 consensus sequence from the Los Alamos database for Subtype C virus. In addition, approximately 20 comparable sequences from Malawi were used, generated as part of another study, to confirm conclusions about sequence variation. Several isolates that were close to the South African consensus sequence were compared to other isolates in distance measurements. Among these 30 isolates, one was chosen as the source for the 30 *gag* gene (SEQ ID NO:4; corresponding to the amino acid sequence in SEQ ID NO:5)

for the following reasons.

This isolate had greater than 95% amino acid identity to the South African consensus sequence, representing the approximate middle of the sequence diversity of 5 all isolates. This isolate, known as DU422, came from a recent seroconvertor, reflecting currently circulating strains and the transmitted phenotype. The phenotype of DU422 is NS1, CCR5(+), and CXCR4(-).

Prior to the insertion of the *gag* gene into the VEE replicon plasmid vector, the 10 amino terminal myristylation (“myr”) site of *gag* was removed to prevent the formation of Gag-containing virus-like particles. Restriction enzyme digests of the *gag* gene plasmid, the capsid helper plasmid, and the glycoprotein helper plasmid were performed to confirm the identity of the three vectors when compared to published maps of the parental plasmid pBR322, with the kanamycin resistance gene substituted 15 for the ampicillin resistance gene. The confirmed plasmid maps of the VEE replicon plasmid containing the DU422 *gag* gene (p3-40.1.6), the capsid helper plasmid (p3-13.2.2), and the glycoprotein helper plasmid (p3-13.4.6) are presented in Figures 1, 2, and 3, respectively. The full nucleotide sequence of each of these plasmids is presented herein as SEQ ID NO:1, SEQ ID NO:8, and SEQ ID NO:11, respectively.

20

In Figures 6 and 15, expression of this HIV-1 Gag protein in BHK cells infected with VRPs expressing such a *gag* construct is demonstrated (Figure 6: Western blot, lane 3; Figure 15, immunofluorescence detection). The cells were infected at a multiplicity of infection (m.o.i.) of 3.5 infectious units (i.u.) per cell, and expression 25 was measured 18 hours post-infection (p.i.). Cell lysates (from approximately 2×10^3 cells) were collected and fractionated either by a 4-12% gradient SDS-PAGE or by 10% SDS-PAGE. The fractionated polypeptides were transferred to PVDF membranes and probed with human HIV-1 positive serum.

30

2. HIV-1 Clade C *env* gene

A Clade C *env* gene (aka "gp160") from another HIV isolate, DU151, from a recent seroconverter was chosen based on its 92% amino acid identity to the South African consensus sequence for this gene, determined in an analogous method to the one described for the *gag* gene in Example 5.A.1. The phenotype of the DU151 isolate is NS1, CCR5(+), CXCR4(-). This gene was engineered into a VEE RNA replicon plasmid as shown in Figure 5, and the entire sequence of the plasmid is given at SEQ ID NO:17. The *env* gene construct used in this Example is SEQ ID NO:18.

10

In Figure 6, expression of this ENV protein (SEQ. ID. NO:19) in BHK cells infected with VRPs expressing this HIV *env* construct is demonstrated (Western blot, lane 2), showing that the protein expressed in the cells is of the correct size and is immunoreactive. In Figure 7, expression of this ENV protein in U87.CD4.CCR5 cells 15 is shown. These cells process the ENV protein into two components, GP120 and GP41. In these cells, the expressed GP160 is fusogenic (see Figure 8).

3. HIV-1 Clade C *pol* gene

20 A Clade C *pol* gene from isolate DU151 was chosen based on its 99% amino acid identity with the South African consensus sequence. This gene was modified at the active site of the reverse transcriptase encoding sequence to inhibit its activity, and the p51 fragment of this modified gene (SEQ ID NO:15) was engineered into a VEE RNA replicon plasmid. The map of this *pol* plasmid is shown in Figure 4, and the 25 nucleotide sequence of the plasmid is provided as SEQ ID NO:14. In Figure 6, expression of this POL p51 fragment (SEQ ID NO:16) in BHK cells is demonstrated (Western blot, lane 1), showing that the protein expressed in these cells is both the correct size and immunoreactive.

30

C. IMMUNOLOGICAL RESPONSE TO VRP-GAG VACCINE

Mice were injected subcutaneously in two doses, with 8-9 mice in each group. The mice were immunized once, then immunized a second time, with the same dose, 28 days later. Serum was collected the day prior to the first immunization, then at day 27 ("after 1st immunization) and at day 35 (after 2nd immunization).

The vigorous, antigen-specific humoral response of mice to the HIV-1 Clade C VRP-gag vaccine described in Example 5.A.1. is presented in Table 1. Details of this assay are described in Example 7A.1.

TABLE 1. Humoral Response to VRP-gag Vaccine

		Total Ab Titer (log ₁₀)
15	Dose:	
	10 ³ i.u. dose:	
	after 1 st immunization	1.3 +/- 0.1
	after 2 nd immunization	2.8 +/- 1.1
20	10 ⁵ i.u. dose	
	after 1 st immunization	2.1 +/- 0.5
	after 2 nd immunization	4.1 +/- 0.6

The vigorous, antigen-specific CTL response in mice to the HIV-1 Clade C VRP-gag vaccine (Example 5.A.1) is presented in Figure 9. Details of this assay are described in Example 7A.3.

EXAMPLE 6
MANUFACTURING PROCESS FOR HIV VRP VACCINES

A. Manufacturing Process

5

Disclosed herein is a manufacturing process for VRP vaccines that is suitable for large-scale preparation of GMP-compliant (GMP = Good Manufacturing Practices) material for use in human clinical trials or for commercial manufacture. The process includes several steps and after each step (as appropriate), a set of "in process control" (IPC) assays or Release Tests (RT) is performed to confirm the successful completion of the step. The process steps and the accompanying IPC assay(s) or RTs (described in more detail in Example 6D.1 and 6D.2) are as follows:

	Process Step	IPC/RT
15	Linearize 3 DNA plasmids	IPC: Check for linearity
	<i>In vitro</i> RNA transcription	IPC: Size, integrity and concentration
	Electroporation of certified Vero cell line	IPC:
	Harvest culture fluids	Titration/Identity Test for replication-competent virus (RCV)
	Pool the culture fluid	RT: Mycoplasma Adventitious virus
20	Purification of bulk VRP by heparin affinity chromatography	PERT assay IPCs: Heparin residual assay BSA assay Bovine IgG assay

Filtration of bulk VRP	RT: Test for RCV Titration/Identity Contaminating protein/DNA Sterility
Formulate, Fill, Release	RT: Titration/Identity Sterility General Safety

B. Preparation of plasmid DNAs

5 Stock solutions of replicon plasmid DNA, capsid helper plasmid DNA and glycoprotein helper plasmid DNA are produced in *Escherichia coli* XL2 Blue cells (Stratagene, cat# 200150). All plasmids harbor the kanamycin resistance gene marker. The three plasmid DNAs were manufactured and purified by PureSyn, Inc. (Malvern, PA) under appropriate GLP/GMP procedures, with a complete Batch Record with full
10 traceability. Following fermentation and cell harvest, cell paste was lysed with base and plasmid DNAs were purified by ion pair chromatography on PolyFloTM separation media.

Prior to release by appropriate quality assurance/quality control oversight, each
15 lot of each plasmid DNA is analyzed to confirm identity, purity and quality (Table 2). An approved certificate of analysis for each DNA is then established for each plasmid DNA lot.

Table 2. Plasmid DNA Release Tests

	Test	Method	Specification
5	DNA homogeneity	Agarose gel electrophoresis	>90% supercoiled
10	<i>E. coli</i> genomic DNA	Southern Blot	<.50 µg/mg plasmid
15	<i>E. coli</i> RNA	Agarose gel electrophoresis	No detectable bands
	Endotoxin	Limulus Amoebocyte Lysate (LAL)	< 0.1 EU/mg
	Total protein	Abs 260/280	1.8-1.9
	Sterility	Bioburden assay, USP23	< 1 CFU
	Identity	Restriction enzyme analysis	Matches map

20 To produce HIV-VRP vaccine for clinical use, both replicon and helper plasmids are linearized by digestion at the unique Not I site and used as templates for synthesis of run-off transcripts. The quality of the transcription products (i.e., the replicon and the two helper RNAs) is evaluated by agarose gel electrophoresis.

25 C. Characterization of the Vero cells

Vero cells are used in the production of HIV-VRPs (WHO Vero MCB P139, BioReliance Inc., Rockville, MD). Vials contained approximately 1×10^7 cells/mL in a cryoprotectant solution of 90% fetal bovine serum and 10% dimethyl sulfoxide. A Cell Certification Summary is provided with each lot. BioReliance Inc. has filed a Master File with the FDA regarding the WHO Vero MCB P139.

Vials of WHO Vero MCB P139 cells are expanded into flasks. Each of the flasks is then expanded again in order to prepare the Master Cell Bank (MCB). The Working Cell Bank (WCB) is prepared from the MCB. The MCB is tested for purity and identity. The WCB is tested for adventitious agents (detection of mycoplasma and viruses). Viability tests are performed on both the MCB and the WCB.

Tumorigenicity tests are performed once at the end of the production period.

D. Electroporation

10

Vero cells are cotransfected by electroporation with RNA mixtures comprising replicon RNA transcripts encoding HIV-gag, VEE capsid helper RNA transcripts, and VEE glycoprotein helper RNA transcripts. The transfected cells are transferred to tissue culture vessels and incubated in well-defined culture medium. Following harvest, the 15 HIV-Gag-VRP is purified from pooled culture fluid supernatants by affinity column chromatography. Prior to formulation and filling, purified, bulk HIV-Gag-VRP is tested for the presence of RCV.

E. Final formulated product

20

The HIV-Gag-VRP vaccine is vialled at four different doses. The material is filtered (0.22 µm) and added to vials at the appropriate concentration and volume, stoppered, quick-frozen and stored at -20° C.

25 **F. Control tests of the Gag-VRP vaccine**

1. In-Process Controls

Table 3 below summarizes the In-Process Controls performed during the 30 manufacturing process of the HIV-Gag-VRP Vaccine.

Table 3. IPCs during the manufacture of HIV-Gag-VRP Vaccine

	Test	Method	Target
5	Check for linearity	Agarose Gel electrophoresis	Report
10	Size, integrity and concentration of RNAs	Agarose Gel electrophoresis	Report
15	Titration/Identity	Indirect immunofluorescence assay(IFA), using standardized Gag-specific antibody preparation	Report
20	Test for RCV	CPE Assay	Report
	Heparin Residual	Chromogenic Inhibitory Assay	Report
	BSA residual	ELISA	Report
	Bovine IgG Residual	ELISA	Report

2. Release tests

Tables 4 and 5 below summarize the release tests performed on the HIV-Gag-VRP Vaccine.

Table 4. Pool of the Culture Fluids

Test	Method	Target
Adventitious Virus (<i>in vivo</i>)	European guidelines	Negative
Adventitious Virus (<i>in vitro</i>)	5 cell lines	No growth
Mycoplasma	21CFR 610.30	No Growth
Reverse Transcriptase	PERT Assay	Negative

Table 5. Bulk VRP and Final Vial testing

Test	Method	Target Result
Replication competent virus (RCV)	Cytopathic effect (CPE) assay	Absence (in BHK cells, sensitivity is 1-10 pfu V3014)
VRP identity/ potency	Indirect immunofluorescence assay (IFA)	10^6 to 10^8 i.u. per mL
Cellular Protein Contaminant	Bio-Rad® DC protein assay	Total protein content per dose
Cellular DNA Contaminant	Southern Blot or PCR	< 10 ng per dose
Sterility	21 CFR § 610.12	Pass

	Endotoxin	LAL	< 5 EU/dose
5	General Safety	21 CFR § 610.11	Pass
	Particulates	USP	Pass
	Stability	IFA	10^6 to 10^8 i.u. per mL

10

EXAMPLE 7
PRECLINICAL STUDIES

Pilot lots are manufactured following written procedures (SOPs and STMs) and according to the manufacturing scheme described in Example 6. These pilot lots are 15 prepared and used for two major tasks. The first one is a preclinical immunogenicity evaluation, which includes studies to assess the immune response and the cell-mediated immune response in vaccinated animals. The second major task is a preclinical safety evaluation, which includes evaluations of system toxicity, hematopoietic and immune system toxicity, and local reactogenicity.

20

Finally, an *in situ* hybridization study is performed in mice in order to verify the *in vivo* expression of HIV-Gag-VRP gene product in lymphoid tissue.

A. Immunogenicity Studies

25

A.1 Humoral Immune Response in Mice

Three groups of five female BALB/c mice (4-6 weeks of age) are inoculated subcutaneously with 10^5 , 10^6 , or 10^7 i.u. of the HIV-Gag-VRP at three time points: on 30 day 0, and at weeks 4 and 8. The fourth group, Control Group, receives the vehicle only. Immediately prior to inoculation, and at weeks 3, 5, 8 and 10 post-inoculation,

blood samples are collected for humoral immune response evaluations. Gag protein-specific serum antibody titers and seroconversion rates are measured by ELISA (Caley *et al.*, 1997) against purified, recombinant Gag protein. The source of the antigen is the homologous Clade C gag gene expressed in insect or mammalian cells. Antigen specificity also is confirmed by immunoblot analysis. Anti-VEE responses are monitored by ELISA (Johnston and Smith, 1988).

A.2 Humoral Immune Response in Rabbits

10 Three groups of five female New Zealand white rabbits are inoculated subcutaneously with 10^5 , 10^6 , or 10^7 i.u. of the HIV-Gag-VRP. The fourth group, Control Group, receives the vehicle only. Immediately prior to inoculation, and at weeks 3, 5, 8 and 10 post-inoculation, blood samples are collected for humoral immune response evaluations.

15

Humoral immune responses are evaluated as described in Section A.1.

A.3 Cell-Mediated Immune Response in Mice

20 Three groups of five female BALB/c mice are inoculated subcutaneously with 10^5 , 10^6 , or 10^7 i.u. of the HIV-Gag-VRP at day 0 and day 28. The fourth group, Control Group, receives the vehicle only. Blood samples are collected at week 3 post-inoculation. Spleens are harvested for splenocyte collection on day 7 following the second inoculation for evaluation of cell-mediated immune responses.

25

The cell-mediated immune response is evaluated by determining the ability of splenic T cells from immunized mice to proliferate *ex vivo* in the presence of either Gag protein or Gag peptide(s). The ability of splenic T and CD4+ T cells to produce interferon- γ and interleukin-4 respectively, is determined. Finally, the ability of 30 cytotoxic T lymphocytes to lyse target cells that present murine major

histocompatibility complex class-I restricted epitopes for HIV-1 Clade C Gag protein is measured (see Betts *et al.*, 1997 for methods)

B. Safety Study

5

Three groups of six male and six female New Zealand white rabbits are inoculated subcutaneously with 10^4 , 10^6 , or 3×10^7 i.u. of the HIV-Gag-VRP. The fourth group, Control Group, receives the vehicle only. Animals receive four injections at week 0, week 3, week 6 and Week 9. Half of the animals are sacrificed two days 10 after the last injection (week 9) and the other half at three weeks after the last injection (week 12). Similar studies are performed in mice with a high dose at 10^8 i.u. This level is 100 times the likely primate dose, based on efficacy studies in rhesus macaques.

In addition to system toxicity (record of mortality/morbidity, body temperature, 15 body weight, food consumption and ophthalmic examinations), hematopoietic toxicity is evaluated by quantitating cellular components of peripheral blood, and immune system toxicity is assessed by histopathologic evaluation of the lymphoid organs. Local reactogenicity is evaluated by examining the injection sites grossly and microscopically to determine irritation potential. Serum samples are also tested for the 20 presence of replication competent virus by blind passage in cell culture.

C. In Situ Hybridization Study in Mice

Three groups of five female BALB/c mice are inoculated subcutaneously with 25 10^5 , 10^6 , or 10^7 i.u. of the HIV-Gag-VRP. The fourth group, Control Group, receives the vehicle only. A single injection is performed in each group.

To verify expression of HIV-GAG-VRP in lymphoid tissue, the draining lymph nodes, spleen, and thymus of the mice are examined by *in situ* hybridization at 24 hours 30 and 48 hours after the single inoculation.

EXAMPLE 8**Heparin Affinity Chromatography of VRPs**

5 Generally, the majority of contaminating protein is non-VEE protein from the conditioned media. Heparin column capacity requirements for GMP manufacturing runs are therefore based on the volume of conditioned media, rather than the concentration of VRPs. Column parameters are optimized at room temperature, but variations in temperature do not greatly affect performance. The expected yields of
10 VRPs can range from 50% to > 90%.

While only minimal leaching of heparin from the columns has been detected, GMP requirements stipulate that a residual heparin assay be performed as an IPC test following the chromatography step.

15

A. Pharmacia HiTrap® Heparin

Five mL columns of Pharmacia HiTrap® Heparin (cat no. 17-0407-01, Amersham Pharmacia Biotech), pre-equilibrated with 25 mM HEPES/0.25 M NaCl, pH
20 7.5, were loaded with HIV-Gag-VRPs produced in Vero cells. After column washing with the equilibration buffer, VRPs were eluted with a 15 column volume gradient from 0.25 – 1.0 M NaCl gradient in 25 mM HEPES, pH 7.5. The HIV-Gag-VRPs eluted at a conductivity of approximately 48 mS/cm. The wash step was optimized (based on the A₂₈₀ peak) at a NaCl concentration between 0.25 M and 0.3 M.

25

B. Heparin Sepharose 6 Fast Flow® resin

Heparin Sepharose 6 Fast Flow® resin (catalog no. 90-1000-2; Amersham Pharmacia Biotech) is supplied as a bulk resin which allows various size columns to be
30 packed as needed. Fast Flow® resins have the advantages of excellent flow

characteristics and ability to be sanitized with sodium hydroxide solutions, which are particularly useful in a GMP manufacturing process. A 6 mL column was prepared by packing the Heparin Sepharose 6 Fast Flow® bulk resin in a BioRad® Econo-Column chromatography column, which was then pre-equilibrated with 25 mM HEPES/0.12 M NaCl, pH 7.5. VRPs were loaded onto the column, which was then washed with the equilibration buffer. Initial experiments indicated that the VRPs eluted at a lower conductivity (36 mS/cm) with this resin as compared to the HiTrap® Heparin, so the wash conditions were modified accordingly. The VRPs were eluted from the Fast Flow® resin with a 15 column volume gradient from 0.12 M to 1 M NaCl in 25 mM HEPES, pH 7.5.

EXAMPLE 9

Virosome Formation

15

The feasibility of virosome formation is demonstrated in a series of experiments in which replicon RNA and RNA encoding the glycoprotein E1 and E2 genes (glycoprotein helper) were first transfected into BHK cells by electroporation. After 18-24 hours, cell supernatants were harvested and tested for the presence of virosomes 20 as described briefly below.

Cell Culture

BHK cells were used as a cell substrate and were maintained in growth medium 25 (alpha-MEM (Life Technologies), supplemented with 10% Fetal Bovine Serum (HyClone), 1x Glutamine (Life-Technologies)), in an atmosphere of 5% CO₂ at 37°C. Prior to electroporation, cells were detached from the cell culture vessel using 0.05% trypsin-0.53 mM EDTA solution (Life Technologies). Trypsin was neutralized with growth medium, and cells were washed twice with cold Phosphate-Buffered Saline 30 (PBS, BioWhittaker) and resuspended at a concentration of 1.5 x 10⁷ cells/ml.

RNA Transcription, Electroporation and Virosome Harvest

Plasmid DNA pVR-GFP (green fluorescent protein) was linearized using restriction endonuclease NotI (New England Biolabs) as recommended by the manufacturer. DNA was extracted with phenol:chloroform:iso-amyl alcohol (25:24:1, Gibco BRL) and precipitated with ethanol, following the addition of NH₄Ac to 2.5 M final concentration. RNA was synthesized in an *in vitro* transcription reaction using an mMessage mMachine® kit (Ambion) as recommended by the manufacturer. This RNA, without further purification, was used to transfect BHK cells. Helper RNA was prepared in a similar fashion. A BHK cell suspension in PBS (0.8 mL, 1.2 x 10⁷ cells) was mixed with 10 µg of each RNA, and the mixture was electroporated. Electroporation settings for Gene-Pulser® (Bio-Rad Laboratories) were: 850 V, 25 µF, 3 pulses. Culture supernatant was collected at 18-24 hr post-electroporation and clarified by centrifugation for 10 min at 1000 rpm.

15

Titration of Virosomes

The presence of infectious virosome particles was demonstrated using an immunofluorescence assay to titer the virosomes by detecting the fluorescence of the GFP encoded by the replicon RNA in the virosomes. Serial dilutions of the cell culture supernatant were added to 12-well plates of BHK cells. Following an 18-24 hour incubation in an atmosphere of 5% CO₂ at 37°C, the medium was removed from each plate. Virosome infectious titer was then determined by counting the number of green-fluorescent single cells at a particular dilution, followed by a back-calculation to determine total infectious units (i.u.) per mL. A final titer of 440 i.u./mL was collected.

Confirmation of virosome identity

Three independent experimental methods were used to determine that the infectious particles were in fact virosomes, rather than replication competent viral

30

particles or naked RNA being carried over from the electroporated cells.

- i) The virosome-containing supernatant was passaged a second time by removing the cell supernatant from the 12-well plate used for titration and placing this supernatant onto a fresh monolayer of BHK cells. At 18-24 hours post-passage, the monolayer was examined under U/V fluorescence and found to contain 0 (zero) GFP-positive cells, indicating the infectious particles produced using this method can undergo only a single round of replication, a critical characteristic of a virosome.
- ii) To establish that the infectious titer detected following virosome packaging was not due to carry-over of RNA used in the electroporation, the supernatant was treated with RNase A (Invitrogen) at a concentration of 100 μ g/ μ L for 15 minutes at 37°C. The treated and untreated control supernatants were titered according to the methods outlined above. The RNase-treated sample contained 400 i.u./mL and the control group had 440 i.u./mL, indicating that the RNase treatment had no significant effect on virosome titer.
- iii) To establish that the infectious particles were enveloped in the E1 and E2 glycoproteins, anti-VEE mouse serum was used to treat the cell supernatant in a neutralization assay. As a control, normal mouse serum was used to treat the virosome supernatant. In addition, VEE replicon particles expressing GFP were used in the assay, the infectivity of which is known to be inhibited by this serum.

		Particle Titer (i.u./mL)		
		Anti-VEE serum	Normal Mouse	No ser
			Serum	
25	Virosome Supernatant	20	440	530
	VRP-GFP	0	530	890

30 The infectivity of the virosomes was inhibited similar to that of VRP-GFP,

indicating that the virosome particles were enveloped by the E1 and E2 glycoproteins.

These examples clearly demonstrate the ability to produce infectious virosome particles comprising replicon RNA enveloped with only the alphavirus E1 and E2 glycoproteins. Testing confirmed that these virosomes are infectious agents, but that they undergo only a single round of replication, as indicated by the inability to passage the agent. In addition, the agents contained the E1 and E2 glycoproteins, as evidenced by the ability to block infection with only VEE specific serum. Finally, the infectious RNA is protected from RNase enzymatic digestion, indicating an enveloped particle.

10

The natural lipid content in BHK cells is primarily non-cationic. Virosomes made in a completely cell free system can be made by using one or more non-cationic lipids, such as lecithin (phosphatidylcholine).

15

EXAMPLE 10
PHASE I CLINICAL PROTOCOL

Phase I Safety and Immunogenicity Trial of an HIV Subtype C Gag-VEE

20 Replicon Particle Vaccine in HIV-1 Seronegative Human Subjects

A Phase I trial is conducted to evaluate the safety and immunogenicity of the HIV Gag-VRP prototype vaccine component in healthy seronegative adult volunteers. The doses are selected based on preclinical studies in rodents and nonhuman primates. 25 The schedule mimics previous preclinical efficacy studies with the SIV model that demonstrated the capacity of SIV-VRP to induce SIV specific neutralizing antibodies and CTL.

Purpose: To evaluate the candidate vaccine component in an open-labeled, 30 placebo-controlled study.

Subjects: Healthy adult volunteers without a history of identifiable high-risk behavior for HIV-1 infection as determined by a comprehensive screening questionnaire.

No. Subjects: 40

5

Route: Subcutaneous injection

Scheme: The volunteers are arranged in four groups, ten subjects per group. In each group, two subjects receive a placebo, while the other eight subjects receive either 10^4 , 10^6 , 10^7 , or 10^8 i.u. of HIV-Gag-VRPs. Subjects are vaccinated on day 0, day 30, and day 120.

Estimated Duration: Forty weeks

A. SELECTION of SUBJECTS

15 Subjects are healthy HIV-1 seronegative adults who fully comprehend the purpose and details of the study as described in the informed consent. Subjects whom either themselves or whose sexual partners have identifiable higher risk behavior for HIV-1 infection are not eligible. Higher risk behavior is determined by a prescreen series of questions designed to identify risk factors for HIV-1 infection. An assessment 20 of absolute exclusion criteria using the self-administered and interview questions is conducted. Subsequently, investigators proceed with phlebotomy, history and physical examination, and final questions regarding sexual behavior and other practices. Eligibility determinations for the trial depend on results of laboratory tests and answers to these self-administered and interview questions.

25

The criteria used to define low risk behavior are as follows:

EITHER ALL OF THE FOLLOWING:

1. No newly acquired higher risk associated STD in the last six months
- 30 2. No possibly safe or unsafe sex with a known HIV+ individual or an active

- injection drug user in the past six months
3. No unsafe sexual activity
 4. Possibly safe sexual activity with two or fewer partners within the last six months
 5. 5. No injection drug use

OR BOTH OF THE FOLLOWING:

1. Mutually monogamous relationship with a known or presumed HIV seronegative partner for the last six months
- 10 2. No injection drug use

A.1 Inclusion Criteria

Age: 18-60

- 15 Sex: Male or Female [*For females, negative pregnancy test at time of entry and assurance that adequate birth control measures will be used for one month prior to immunization and the duration of the study]*]
Normal history and physical examination
Lower risk sexual behavior as defined above.
- 20 Normal complete blood count and differential defined as:
 - Hematocrit 34% for women; 38% for men
 - White count 3500 cells/mm³ with normal differential
 - Total lymphocyte count 800 cells/mm³
 - Absolute CD4 count 400 cells/mm³
- 25 - Platelets (150,000-550,000)
Normal ALT (~ 1.5 x institutional upper normal limit) and creatinine (1.6 mg/dl)
Normal urine dipstick with esterase and nitrite
Negative for hepatitis B surface antigen
- 30 Negative ELISA for HIV within eight weeks of immunization

Availability for follow-up for planned duration of the study (68 weeks)

A viable EBV transformed autologous B cell line

A.2 Exclusion Criteria

5

History of immunodeficiency, chronic illness, malignancy, autoimmune disease, or use of immunosuppressive medications

10 Medical or psychiatric condition or occupational responsibilities which preclude subject compliance with the protocol

Subjects with identifiable higher risk behavior for HIV infection as determined by screening questionnaire designed to identify risk factors for HIV infection; specific exclusions include:

15 History of injection drug use within the last 12 months prior to enrollment.

Higher risk sexual behavior defined as one or more of the following behaviors:

1. A newly acquired higher risk associated STD within the past six months
2. Possibly safe or unsafe sex with a known HIV+ individual in the past six months

20 3. Possibly safe sexual activity with twelve or more partners in the past six months

4. Unsafe sexual activity with four or more partners within the past six months.

25 Live attenuated vaccines within 60 days of study [NOTE: Medically indicated subunit or killed vaccines (e.g., influenza, pneumococcal) are not exclusionary, but should be given at least two weeks away from test article immunizations.]

Use of experimental agents within 30 days prior to study

Receipt of blood products or immunoglobulin in the past six months

30 Active syphilis [NOTE: If the serology is documented to be a false positive or

due to a remote (>six months) treated infection, the volunteer is eligible]

Active tuberculosis [NOTE: Volunteers with a positive PPD and a normal chest X-ray showing no evidence of TB and not requiring INH therapy are eligible.]

History of anaphylaxis or other serious adverse reactions to vaccines

5 Prior receipt of HIV vaccines or a placebo recipient in an HIV vaccine trial

Pregnant or lactating women

B. SAFETY and IMMUNOGENICITY MONITORING

10 Safety is evaluated by monitoring volunteers for adverse reactions during the course of the trial. Volunteers are followed for a total of 26 weeks post-final inoculation. The main toxicity associated with the subcutaneous injection in this study is that associated with subcutaneous injection of any immunogen, i.e., pain, redness and swelling at the injection site, as well as the possibility of fever, chills, aches and pains
15 and perhaps fatigue.

Safety monitoring includes periodic review of data from the trial with particular emphasis on monitoring for adverse reactions including the following evaluations:

20 Hematologic: CBC, differential, platelets

Hepatic/renal: ALT, creatinine, urinalysis

Neurologic: headache, paralysis, anxiety, confusion, weakness, tremors.

Systemic symptoms: fever, gastrointestinal complaints, myalgia, malaise, fatigue, headache, anaphylaxis, immune complex disease, and other hypersensitivity reactions

25 Local toxicity at the site of injection: e.g., pain, tenderness, erythema, regional lymphadenopathy, limitation of limb movement

The immunogenicity monitoring includes the following immunological assays, all utilizing HIV Subtype C based reagents:

Humoral responses:

HIV Subtype C Gag-specific ELISA
Anti-VEE ELISA

5 Cellular immune responses:

Standard cell-killing assay (i.e., chromium release) to measure CD8+ Gag-specific CTL activity

ELISPOT assay to measure IFN-?

10 Mucosal immune responses:

Standardized assay for assessment of Gag-specific IgA

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference
15 into this application in order to more fully describe the state of the art to which this invention pertains.

REFERENCES

- 20 Barany F. 1985. Single-stranded hexameric linkers: a system for in-phase insertion mutagenesis and protein engineering. *Gene* 37(1-3):111-23.
- 25 Betts, M. R., J. Krowka, C. Santamaria, K. Balsamo, F. Gao, G. Mulundu, C. Luo, N. N'Gandu, H. Sheppard, B. H. Hahn, S. Allen and J. A. Frelinger. 1997. Cross-clade human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte responses in HIV-infected Zambians. *J. Virol.* 71:8908-8911.
- 30 Caley, I. J., M.R. Betts, D.M. Irlbeck, N. L. Davis, R. Swanstrom, J.A. Frelinger and R. E. Johnston. 1997. Humoral, mucosal and cellular immunity in response to an HIV-1 vaccine candidate. *J. Virol.* 71:3031-3038.

Davis *et al.* 1980. In: Microbiology, 3d ed., p. 132.

Davis, N.L., L.V. Willis, J.F. Smith and R.E. Johnston. 1989. *In vitro* synthesis of infectious Venezuelan equine encephalitis virus RNA from a cDNA clone: Analysis of 5 a viable deletion mutant. *Virology* 171:189-204.

Davis, N. L., L. V. Willis, J. F. Smith, G. Greenwald and R. E. Johnston. 1990. *In vitro* synthesis of infectious VEE virus RNA from a cDNA clone: Analysis of a viable deletion mutant and mutations affecting virulence. In: Vaccines 90, Cold Spring Harbor 10 Press, Cold Spring Harbor, NY. pp. 109-113.

Davis, N. L., N. Powell, G. F. Greenwald, L. V. Willis, B. J. Johnson, J. F. Smith and R. E. Johnston. 1991. Attenuating mutations in the E2 glycoprotein gene of Venezuelan equine encephalitis virus: Construction of single and multiple mutants in a 15 full-length cDNA clone. *Virology* 183:20-31.

Davis, N. L., K. W. Brown, G. F. Greenwald, A. J. Zajac, V. L. Zacny, J. F. Smith and R. E. Johnston. 1995. Attenuated mutants of Venezuelan equine encephalitis virus containing lethal mutations in the PE2 cleavage signal combined with a second-site 20 suppressor mutation in E1. *Virology* 212:102-110.

Davis, N. L., K. W. Brown and R. E. Johnston. 1996a. A viral vaccine vector that expresses foreign genes in lymph nodes and protects against mucosal challenge. *J. Virol.* 70:3781-3787.

25

Davis, N. L., P. Pushko, K. W. Brown, P. C. Charles, I. J. Caley, M. Parker, G. Ludwig, J. F. Smith and R. E. Johnston. 1996b. Immunization against influenza with attenuated Venezuelan equine encephalitis virus vectors. In: Options for the Control of Influenza, III, L. E. Brown and A. W. Hampson, eds. Elsevier, Amsterdam. pp.803-30 809.

- Davis, N. L., I. J. Caley , K. W. Brown, M.R. Betts, D.L. Irlbeck, K.M. McGrath, M.J. Connell, D.C. Montefiori, J.A. Frelinger, R. Swanstrom, P.R. Johnson and R. E. Johnston. 2000. Vaccination of macaques against pathogenic simian immunodeficiency virus with Venezuelan equine encephalitis virus replicon particles. *J. Virol.* 74:371-378
- 5 Grieder, F. B., N. L. Davis, J. F. Aronson, P. C. Charles, D. C. Sellon, K. Suzuki and R. E. Johnston. 1995. Specific restrictions in the progression of Venezuelan equine encephalitis virus induced disease resulting from single amino acid changes in the glycoproteins. *Virology* 206:994-1006.
- 10 Hevey, M., D. Negley, P. Pushko, J. Smith and A. Schmaljohn. 1998. Marburg virus vaccines based upon alphavirus replicons protect guinea pigs and nonhuman primates. *Virology* 251:28-37.
- 15 Hirsch, V., T.R. Fuerst, G. Sutter, M.W. Carroll, L. C. Yang, S. Goldstein *et al.* 1996. Patterns of viral replication correlate with outcome in SIV-infected macaques: effect of prior immunization with a trivalent SIV vaccine in modified vaccinia virus Ankara. *J. Virol.* 70:3741-3752.
- 20 Johnston, Robert E. and Jonathan F. Smith. 1988. Selection for accelerated penetration in cell culture co-selects for attenuated mutants of Venezuelan equine encephalitis virus. *Virology* 162:437-443.
- Johnston, R. E. and C. J. Peters. 1996. *Alphaviruses*. In: Virology, Third Edition, B. N. Fields, D. M. Knipe and P. M. Howley, eds., Raven Press, New York. pp. 843-898.
- Kinney, R.M., B.J.B. Johnson, J.B. Welch, K.R. Tsuchiya and D.W. Trent. 1989. The full-length nucleotide sequences of the virulent Trinidad donkey strain of Venezuelan equine encephalitis virus and its attenuated vaccine derivative, strain TC-83. *Virology*
- 30 170:19-30.

- Kinney, R.M., G-J. Chang, K. R. Tsuchiya, J. M. Sneider, J. T. Roehrig, T. M. Woodward and D. W. Trent. 1993. Attenuation of Venezuelan equine encephalitis virus strain TC-83 is encoded by the 5'-noncoding region and the E2 envelope glycoprotein. *J. Virol.* 67:1269-1277.
- 5
Kunkel. 1985. *Proc. Natl. Acad. Sci. USA* 82:488.
- Paredes, A.M., D.T. Brown, R. Rothnagel, W. Chiu, R.J. Schoepp, R.E. Johnston and B.V.Prasad. 1993. Three-dimensional structure of a membrane-containing virus. *Proc. 10 Natl. Acad. Sci., USA* 90:9095-9099.
- Pushko, P., M. Parker, G. V. Ludwig, N. L. Davis, R. E. Johnston and J. F. Smith. 1997. Replicon-helper systems from attenuated Venezuelan equine encephalitis virus: expression of heterologous genes *in vitro* and immunization against heterologous 15 pathogens *in vivo*. *Virology* 239:389-401.
- Rosenberg, A.H., *et al.* 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene*. 56(1): p. 125-35.
- 20 Schlesinger, S. and M.J. Schlesinger. 1990. *Replication of Togaviridae and Flaviviridae*. In: Virology, Fields, B.N. and Knipe, D.M. (eds.) Raven Press. pp. 697-711.
- Strauss *et al.* 1990. *Seminars in Virology* 1:347.
25
Strauss, J.H. and E.G. Strauss. 1994. The alphaviruses: Gene expression, replication, and evolution. *Micro. Rev.* 58:491-562.
- Studier, F.W., *et al.* 1990. Use of T7 RNA polymerase to direct expression of cloned 30 genes. 1990. *Methods Enzymol.* 185:60-89.

What is claimed is:

1. A composition comprising two or more isolated nucleic acids selected from the group consisting of an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity.
2. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.
3. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag*

gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle, and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

4. A method of making the population of alphavirus replicon particles of claim 2 comprising:
 - A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
 - (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
 - and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the

helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains

no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells; and
- D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 2.

5. The method of claim 4, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

6. A method of making the population of alphavirus replicon particles of claim 3, comprising:

- A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein;

and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particle, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and

furthermore not encoding at least one other alphavirus structural protein;
and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and

furthermore not encoding at least one other alphavirus structural protein;

and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 3.

7. The method of claim 6, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

8. The method of claim 6, wherein only at least one of the first population of alphavirus particles, the second population of alphavirus particles and the third population of alphavirus particles comprises particles wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations.
9. A population of alphavirus replicon particles produced by the method of claim 4.
10. A population of alphavirus replicon particles produced by the method of claim 6.
11. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 1 in a pharmaceutically acceptable carrier.
12. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 2 in a pharmaceutically acceptable carrier.
13. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 3 in a pharmaceutically acceptable carrier.
14. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 9 in a pharmaceutically acceptable carrier.
15. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the

population of claim 10 in a pharmaceutically acceptable carrier.

16. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 1 in a pharmaceutically acceptable carrier.

17. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 2 in a pharmaceutically acceptable carrier.

18. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 3 in a pharmaceutically acceptable carrier.

19. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 9 in a pharmaceutically acceptable carrier.

20. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 10 in a pharmaceutically acceptable carrier.

21. A composition comprising two or more isolated nucleic acids selected from the group consisting of an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof

of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof.

22. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.

23. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a

modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle, and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

24. A method of making the population of alphavirus replicon particles of claim 22, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells;
- B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;
- (b) producing the alphavirus particles in the helper cell; and

- (c) collecting the alphavirus particles from the helper cells;
- C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;
- (b) producing the alphavirus particles in the helper cell; and
 - (c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 22.

25. The method of claim 24, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

26. A method of making the population of alphavirus replicon particles of claim 23, comprising:

- A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
 - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
 - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particle, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein

- not encoded by said first helper RNA;
and with at least one of said helper RNAs lacking an alphavirus packaging signal;
wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;
- (b) producing the alphavirus particles in the helper cell; and
(c) collecting the alphavirus particles from the helper cells;
- C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional

helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 23.

27. The method of claim 26, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

28. The method of claim 26, wherein only at least one of the first population of alphavirus particles, the second population of alphavirus particles and the third population of alphavirus particles comprises particles wherein at least one of said

replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations.

29. A population of alphavirus replicon particles produced by the method of claim
24.

30. A population of alphavirus replicon particles produced by the method of claim
26.

31. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 21 in a pharmaceutically acceptable carrier.

32. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 22 in a pharmaceutically acceptable carrier.

33. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 23 in a pharmaceutically acceptable carrier.

34. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 29 in a pharmaceutically acceptable carrier.

35. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 30 in a pharmaceutically acceptable carrier.

36. A method of treating or preventing infection by human immunodeficiency

virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 21 in a pharmaceutically acceptable carrier.

37. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 22 in a pharmaceutically acceptable carrier.

38. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 23 in a pharmaceutically acceptable carrier.

39. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 29 in a pharmaceutically acceptable carrier.

40. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 30 in a pharmaceutically acceptable carrier.

41. An alphavirus replicon virosome comprising an alphavirus replicon RNA encapsidated by a lipid bilayer comprising alphavirus glycoproteins, E1 and E2.

42. The viroosome of claim 41, wherein the alphavirus glycoproteins are Venezuelan Equine Encephalitis glycoproteins E1 and E2.

43. A method of producing the alphavirus replicon virosome of claim 41, comprising:

a) combining alphavirus replicon RNA, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced.

44. An alphavirus replicon virosoe produced from the method of claim 43.

45. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon virosoe of claim 41 in a pharmaceutically acceptable carrier.

46. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon virosoe of claim 41, wherein the virosoe comprises alphavirus replicon RNA encoding one or more HIV immunogens.

47. A composition comprising a population of alphavirus replicon virosomes comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon virosoe.

48. A composition comprising a population of alphavirus replicon virosomes

comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation of reverse transcriptase activity in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon virosome.

49. A method of producing the population of alphavirus replicon virosomes of claim 47, comprising:

A) (a) producing a first population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *env* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

B) (a) producing a second population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *gag* gene product or immunogenic fragment thereof, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes

are produced;

C) (a) producing a third population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding the *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced; and

D) combining the first population of alphavirus replicon virosomes, the second population of alphavirus replicon virosomes and the third population of alphavirus replicon virosomes to produce the population of alphavirus replicon virosomes of claim 47.

50. A method of producing the population of alphavirus replicon virosomes of claim 48, comprising:

A) (a) producing a first population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *env* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

B) (a) producing a second population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding and *gag* gene product or immunogenic fragment thereof, wherein the *gag* gene product or

immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

C) (a) producing a third population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding the *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation of reverse transcriptase activity in the *pol* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced; and

D) combining the first population of alphavirus replicon virosomes, the second population of alphavirus replicon virosomes and the third population of alphavirus replicon virosomes to produce the population of alphavirus replicon virosomes of claim 48.

51. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 47, in a pharmaceutically acceptable carrier.

52. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 48, in a pharmaceutically acceptable carrier.

53. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 47, in a pharmaceutically acceptable carrier.

54. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 47, in a pharmaceutically acceptable carrier.

55. A composition comprising heparin affinity-purified alphavirus replicon particles, wherein the alphavirus replicon particles comprise at least one structural protein which comprises one or more attenuating mutations.

56. A method of preparing the heparin affinity-purified alphavirus particles of claim 55, comprising:

- a) producing alphavirus replicon particles, wherein the alphavirus replicon particles comprise at least one structural protein which comprises one or more attenuating mutations;
- b) loading the alphavirus replicon particles of step (a) in a heparin affinity chromatography column; and
- c) collecting the fraction from the column which contains the heparin affinity-purified alphavirus replicon particles.

57. A composition produced by the method of claim 56.

58. A method of producing VRP for use in a vaccine comprising:

- a) producing a plasmid encoding the nucleotide sequence of an alphavirus replicon RNA;
- b) producing a plasmid encoding the nucleotide sequence of one or more helper RNAs;
- c) transcribing the plasmids of steps (a) and (b) into RNA *in vitro*;
- d) electroporating the RNA of step (c) into a Vero cell line; and
- e) purifying VRP from the Vero cell line of step (d) by heparin affinity chromatography.

59. The method of claim 58, wherein the VRP is produced in large-scale.
60. VRP produced by the method of claim 59.
61. An isolated nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof.
62. A composition comprising the nucleic acid of claim 61.
63. A vector comprising the nucleic acid of claim 61.
64. A cell comprising the vector of claim 63.
65. An alphavirus replicon particle comprising the nucleic acid of claim 61.
66. A method of making the alphavirus replicon particle of claim 65, comprising
 - a) providing a helper cell for producing an infectious, defective alphavirus particle, comprising in an alphavirus-permissive cell:
 - (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cell.

67. The method of claim 66, wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations.

68. An alphavirus replicon particle produced according to the method of claim 66.

69. An alphavirus replicon particle produced according to the method of claim 67.

70. A method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 62 in a pharmaceutically acceptable carrier.

71. A method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon particle of claim 65 in a pharmaceutically acceptable carrier.
72. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 62 in a pharmaceutically acceptable carrier.
73. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon particle of claim 65 in a pharmaceutically acceptable carrier.
74. A method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of a composition comprising the alphavirus replicon particles of claim 65 in a pharmaceutically acceptable carrier.
75. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of a composition comprising the alphavirus replicon particles of claim 65 in a pharmaceutically acceptable carrier.

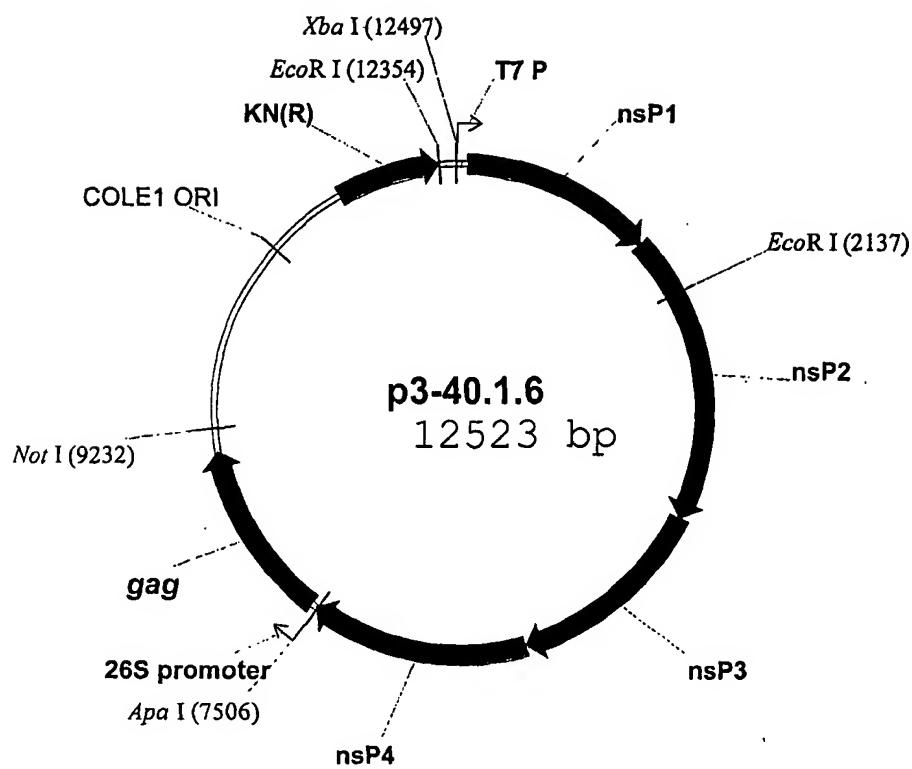
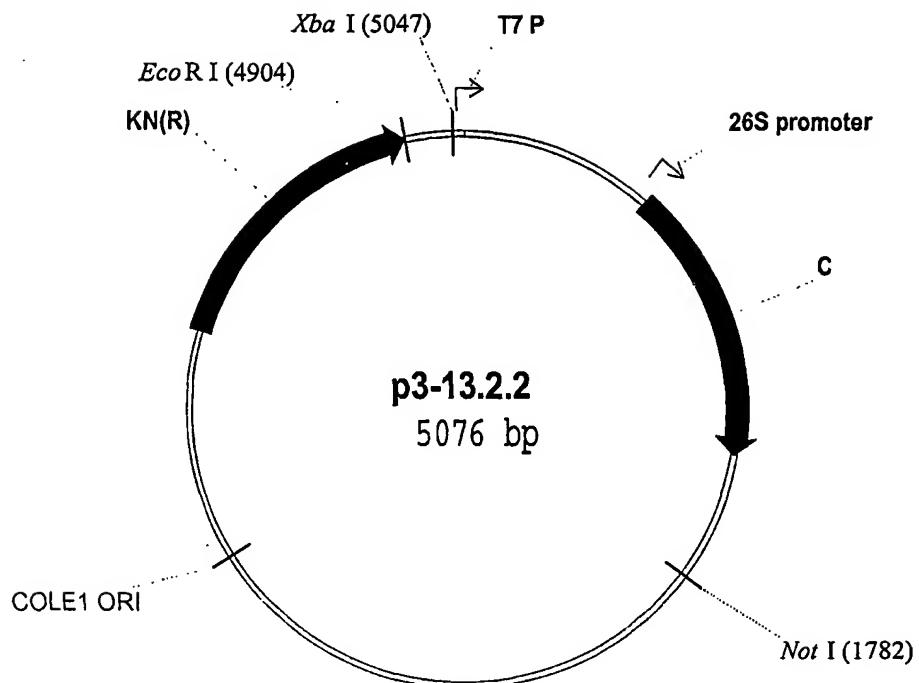


FIG. 1

**FIG. 2**

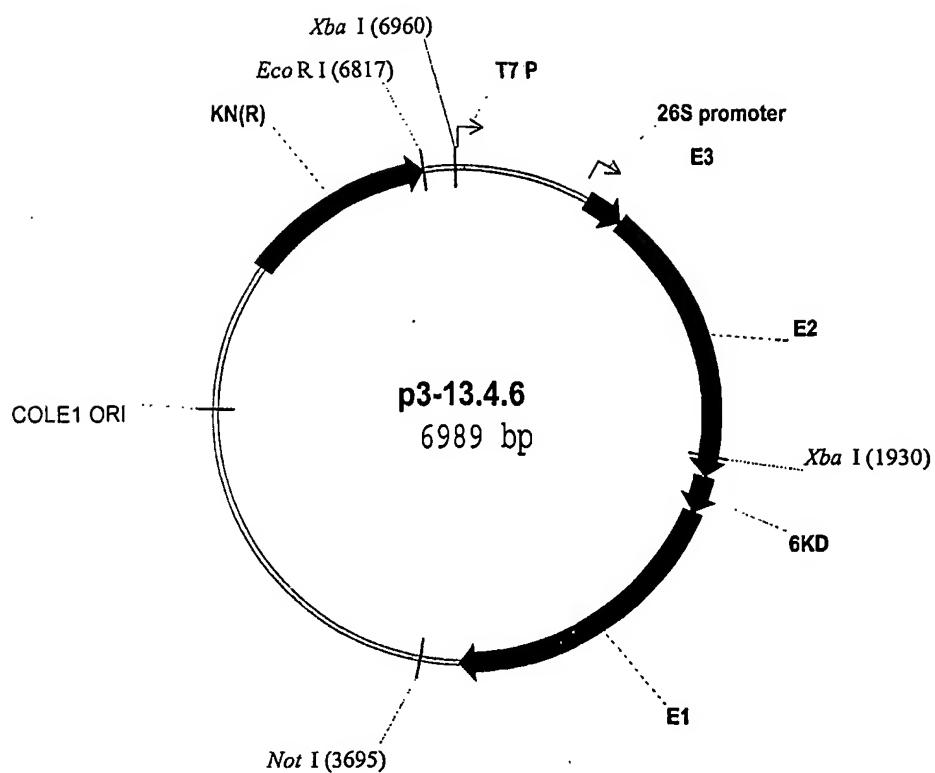


FIG. 3

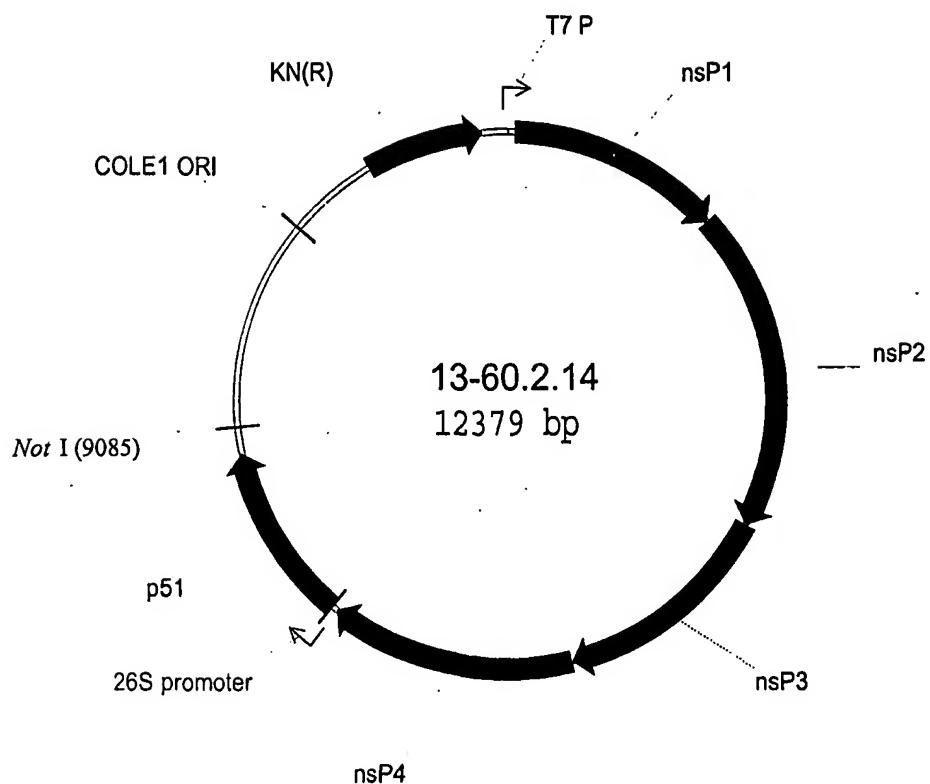


FIG. 4

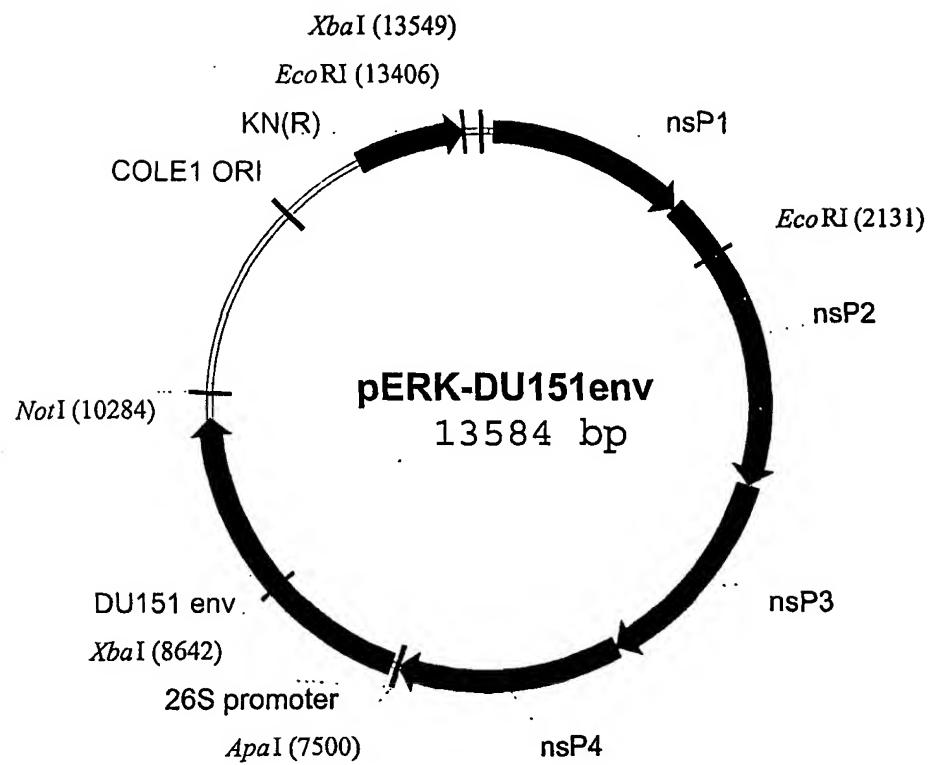
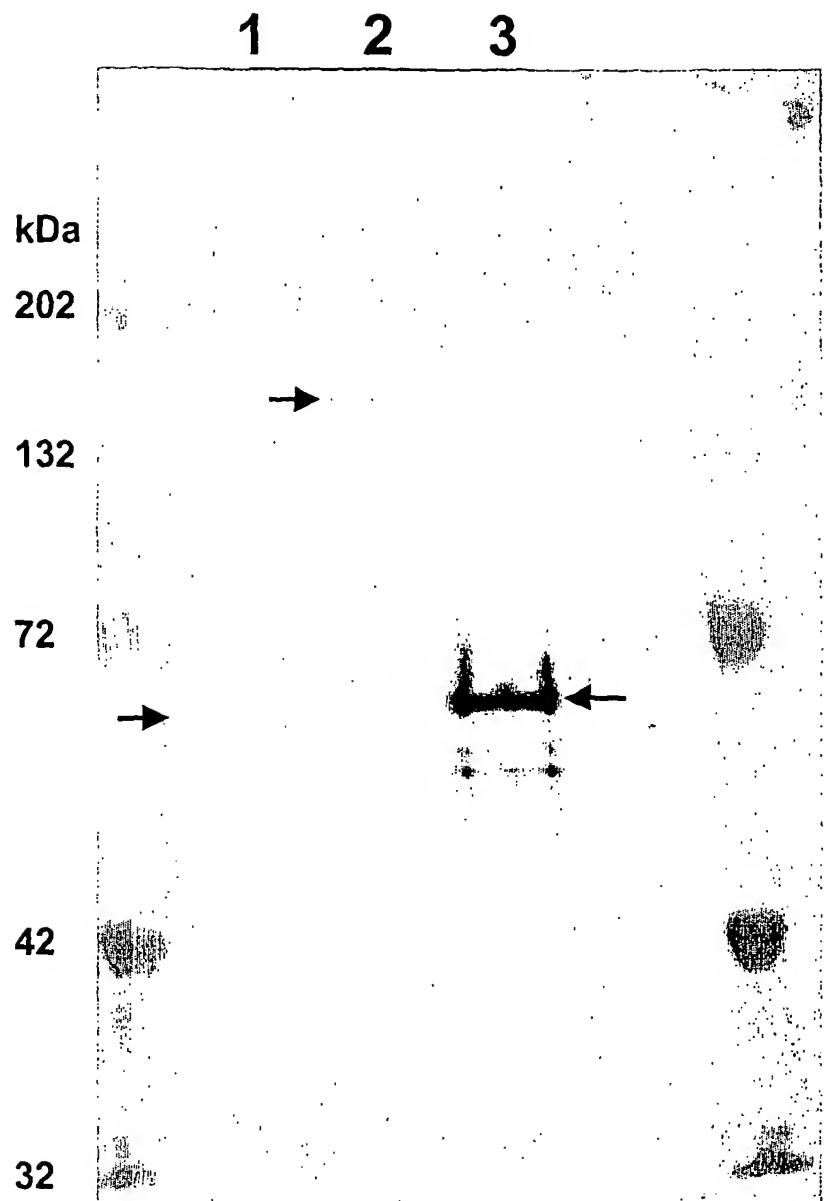


FIG. 5

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**FIG. 6**

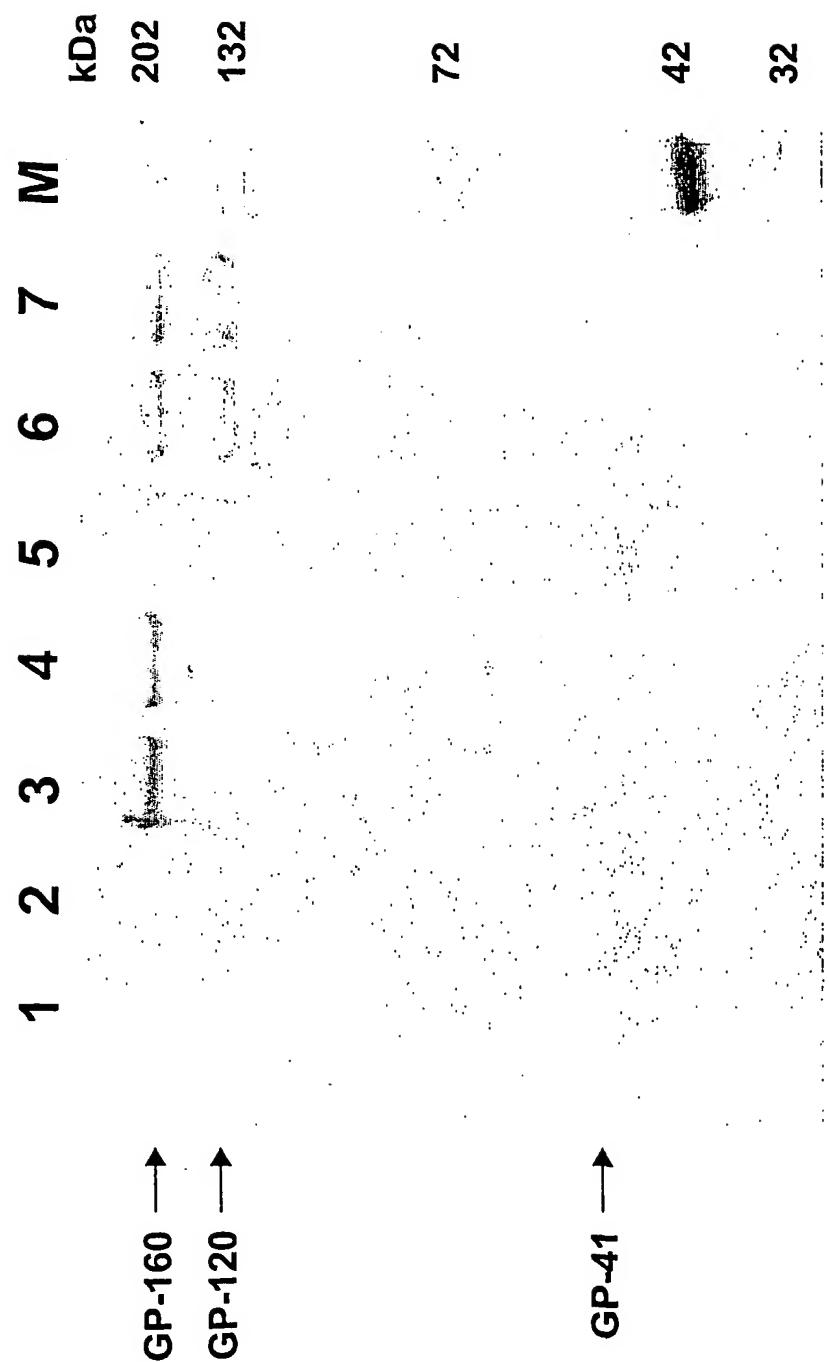


FIG. 7

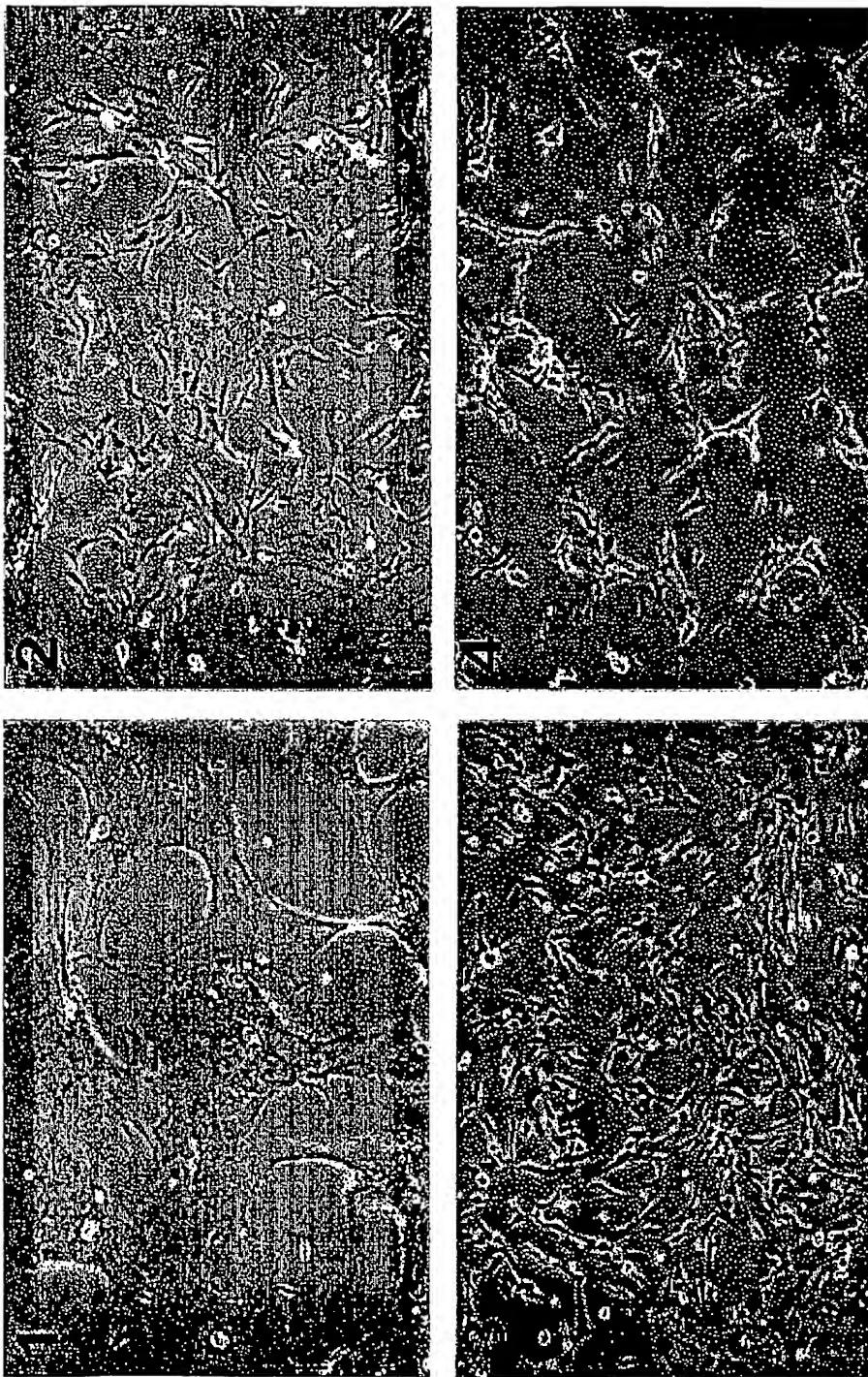
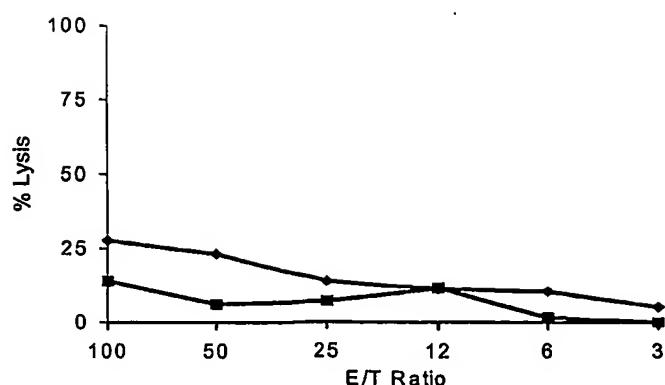
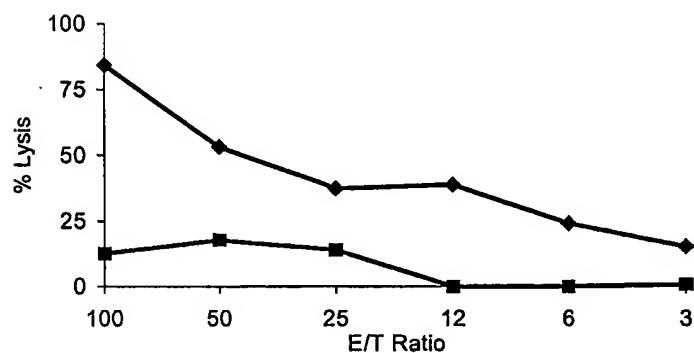
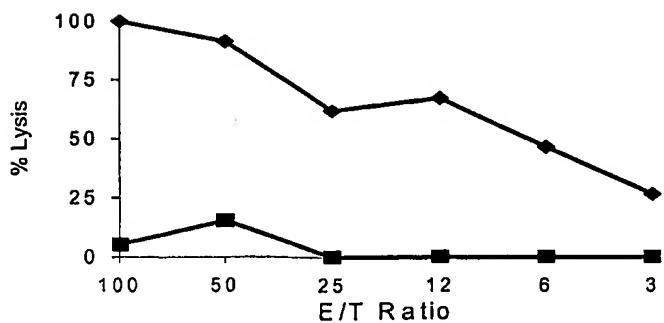


FIG. 8

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**FIG. 9A****FIG. 9B****FIG. 9C**

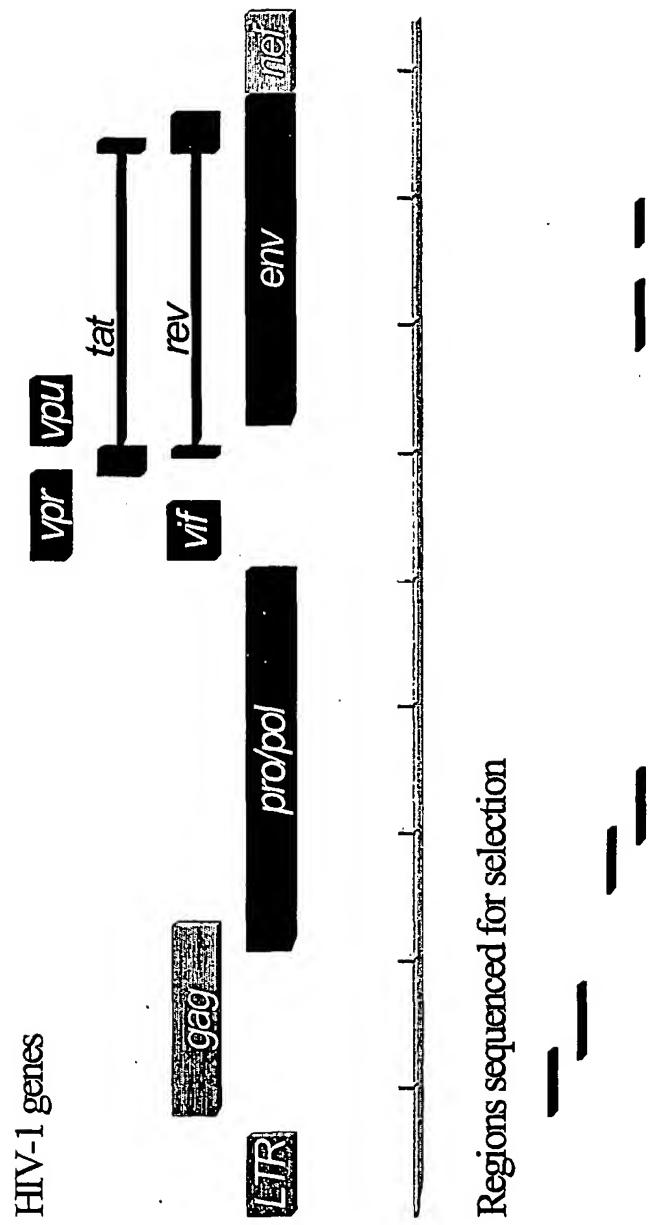


FIG. 10

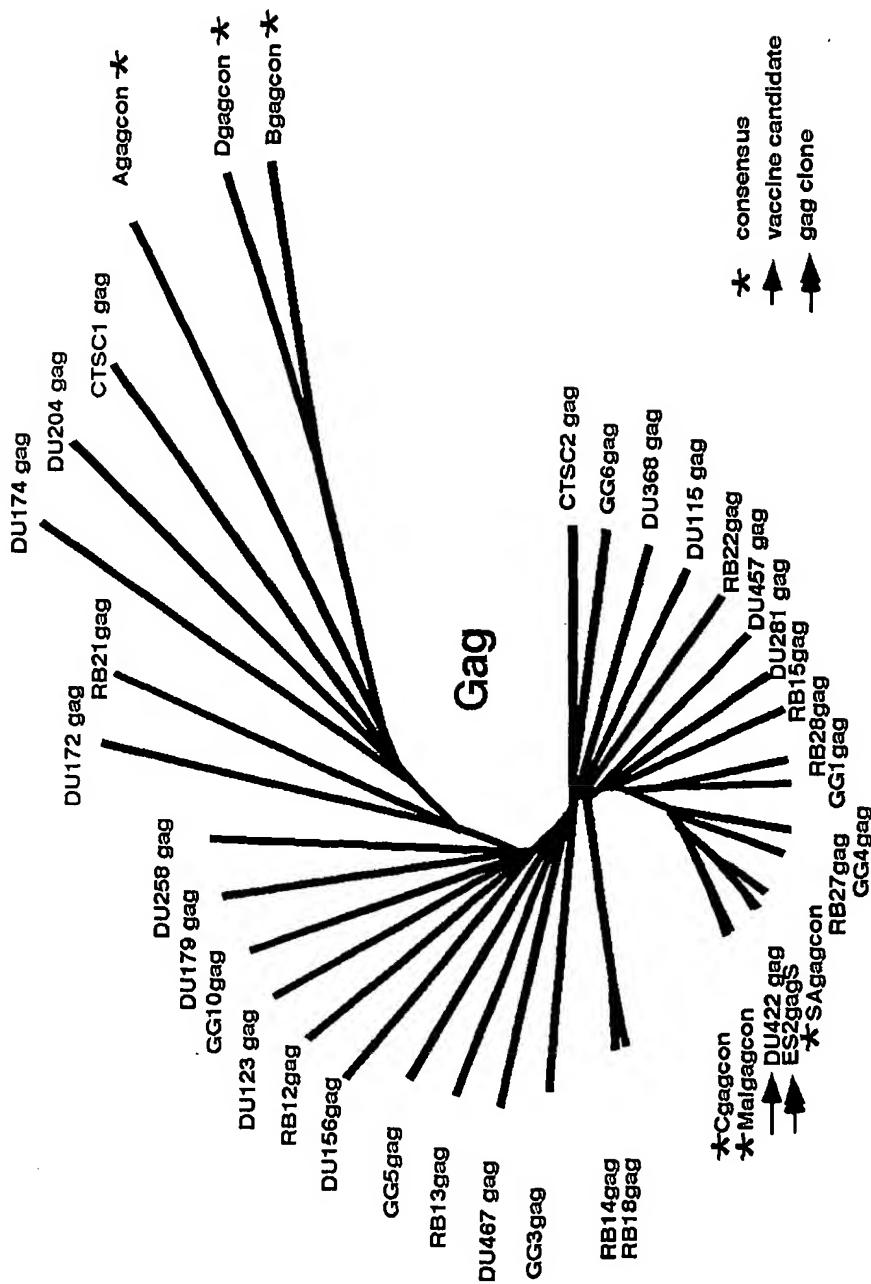


FIG. 11

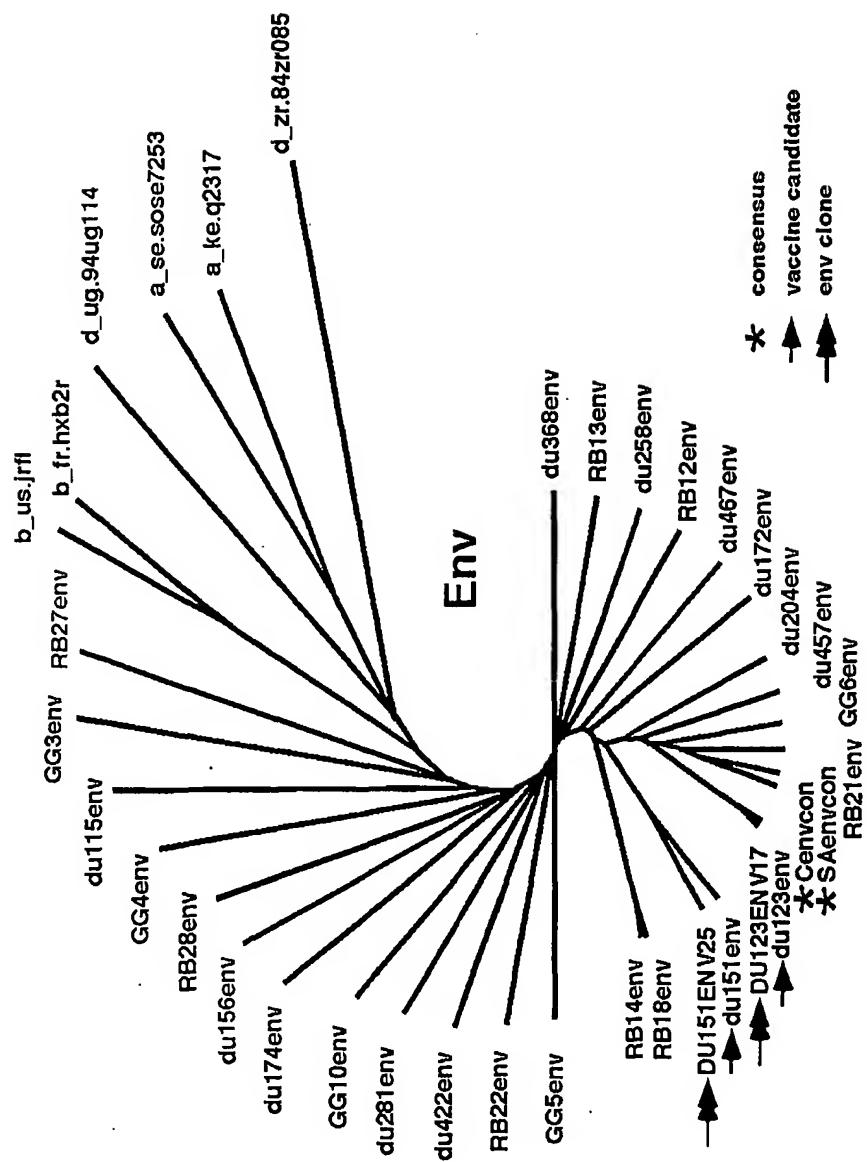
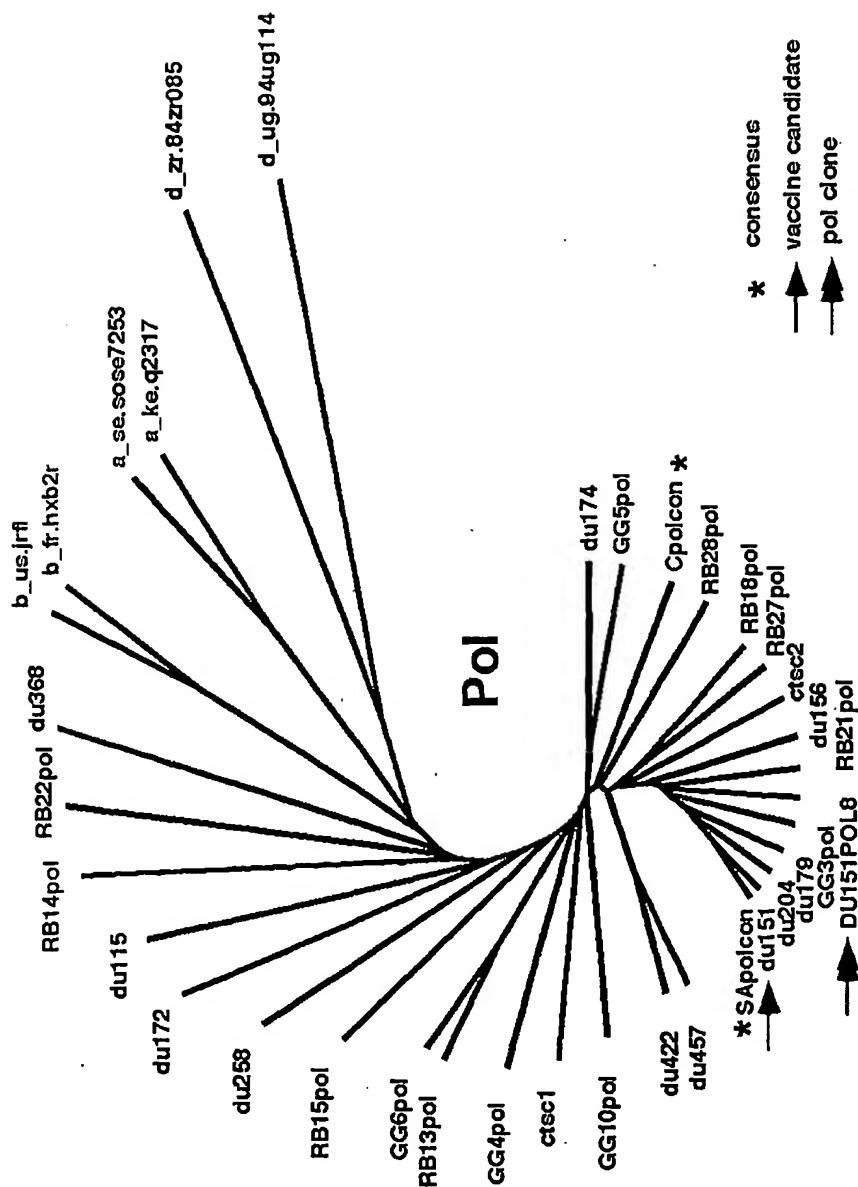
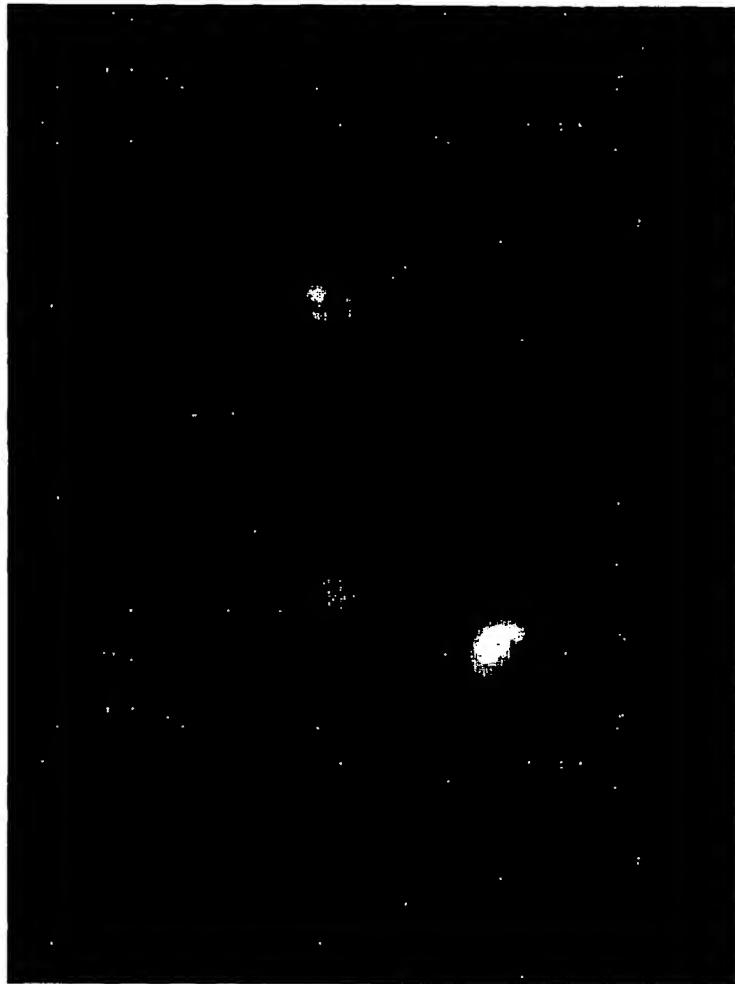


FIG. 12

**FIG. 13**



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FIG. 14

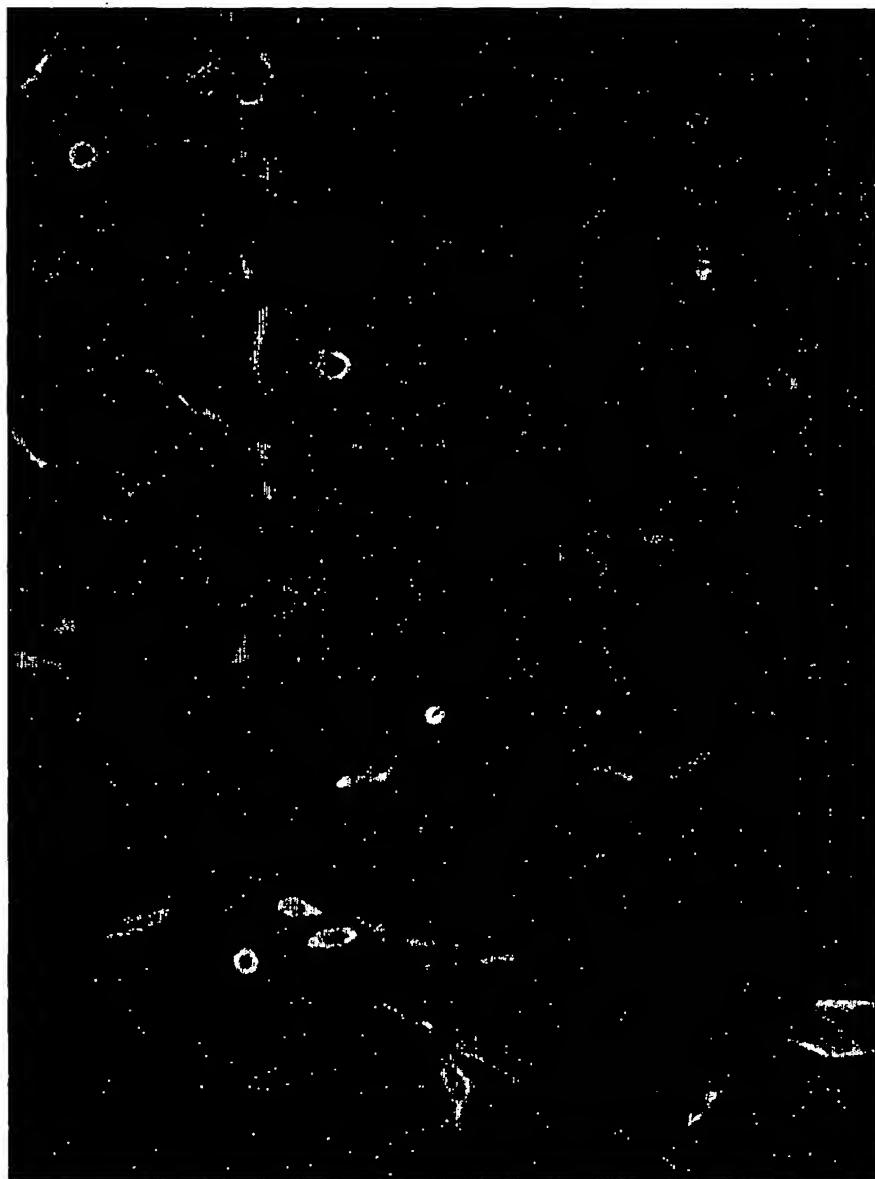


FIG. 15

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Daley, Ian
Maugham, Maureen
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Swanstrom, Ronald

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synthetic construct

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Ala Leu Gln Arg Ser Phe Pro Gln Phe Glu Val Ala Lys Gln Val
20 25 30

act gat aat gac cat gct aat gcc aga gcg ttt tcg cat ctg gct tca 144
Thr Asp Asn Asp His Ala Asn Ala Arg Ala Phe Ser His Leu Ala Ser
35 40 45

aaa ctg atc gaa acg gag gtg gac cca tcc gac acg atc ctt gac att 192
Lys Leu Ile Glu Thr Glu Val Asp Pro Ser Asp Thr Ile Leu Asp Ile
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gca act aag ctg aag aaa aac tgt aag gaa ata act gat aag gaa ttg Ala Thr Lys Leu Lys Asn Cys Lys Glu Ile Thr Asp Lys Glu Leu 100 105 110	336
gac aag aaa atg aag gag ctc gcc gtc atg agc gac cct gac ctg Asp Lys Lys Met Lys Glu Leu Ala Ala Val Met Ser Asp Pro Asp Leu 115 120 125	384
gaa act gag act atg tgc ctc cac gac gag tcg tgt cgc tac gaa Glu Thr Glu Thr Met Cys Leu His Asp Asp Glu Ser Cys Arg Tyr Glu 130 135 140	432
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Arg Ile Ala Ile Ser Pro Gly Leu Tyr Gly Lys Pro Ser Gly Tyr Ala	290	295	300	
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Leu Asn Gly Glu Arg Val Ser Phe Pro Val Cys Thr Tyr Val Pro Ala	325	330	335	
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Thr Leu Cys Asp Gln Met Thr Gly Ile Leu Ala Thr Asp Val Ser Ala	340	345	350	
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Lys Arg Pro Asp Thr Gln Thr Ile Ile Lys Val Asn Ser Asp Phe His	435	440	445	
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Ser Phe Val Leu Pro Arg Ile Gly Ser Asn Thr Leu Glu Ile Gly Leu	450	455	460	
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Arg Thr Arg Ile Arg Lys Met Leu Glu Glu His Lys Glu Pro Ser Pro	465	470	475	
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Ala Lys Glu Val Arg Glu Ala Glu Glu Leu Arg Ala Ala Leu Pro Pro	500	505	510	

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ata agg gac gtc aag aaa atg aaa ggg ctg gac gtc aat gcc aga act Ile Arg Asp Val Lys Lys Met Gly Leu Asp Val Asn Ala Arg Thr 755 760 765	2304
gtg gac tca gtg ctc ttg aat gga tgc aaa cac ccc gta gag acc ctg Val Asp Ser Val Leu Leu Asn Gly Cys Lys His Pro Val Glu Thr Leu 770 775 780	2352
tat att gac gaa gct ttt gct tgt cat gca ggt act ctc aga gcg ctc Tyr Ile Asp Glu Ala Phe Ala Cys His Ala Gly Thr Leu Arg Ala Leu 785 790 795 800	2400
ata gcc att ata aga cct aaa aag gca gtg ctc tgc ggg gat ccc aaa Ile Ala Ile Ile Arg Pro Lys Lys Ala Val Leu Cys Gly Asp Pro Lys 805 810 815	2448
cag tgc ggt ttt ttt aac atg atg tgc ctg aaa gtg cat ttt aac cac Gln Cys Gly Phe Phe Asn Met Met Cys Leu Lys Val His Phe Asn His 820 825 830	2496
gag att tgc aca caa gtc ttc cac aaa agc atc tct cgc cgt tgc act Glu Ile Cys Thr Gln Val Phe His Lys Ser Ile Ser Arg Arg Cys Thr 835 840 845	2544
aaa tct gtg act tcg gtc gtc tca acc ttg ttt tac gac aaa aaa atg Lys Ser Val Thr Ser Val Val Ser Thr Leu Phe Tyr Asp Lys Lys Met 850 855 860	2592
aga acg acg aat ccg aaa gag act aag att gtg att gac act acc ggc Arg Thr Thr Asn Pro Lys Glu Thr Lys Ile Val Ile Asp Thr Thr Gly 865 870 875 880	2640
agt acc aaa cct aag cag gac gat ctc att ctc act tgt ttc aga ggg Ser Thr Lys Pro Lys Gln Asp Asp Leu Ile Leu Thr Cys Phe Arg Gly 885 890 895	2688
tgg gtg aag cag ttg caa ata gat tac aaa ggc aac gaa ata atg acg Trp Val Lys Gln Leu Gln Ile Asp Tyr Lys Gly Asn Glu Ile Met Thr 900 905 910	2736
gca gct gcc tct caa ggg ctg acc cgt aaa ggt gtg tat gcc gtt cgg Ala Ala Ala Ser Gln Gly Leu Thr Arg Lys Gly Val Tyr Ala Val Arg 915 920 925	2784
tac aag gtg aat gaa aat cct ctg tac gca ccc acc tca gaa cat gtg Tyr Lys Val Asn Glu Asn Pro Leu Tyr Ala Pro Thr Ser Glu His Val 930 935 940	2832
aac gtc cta ctg acc cgc acg gag gac cgc atc gtg tgg aaa aca cta Asn Val Leu Leu Thr Arg Thr Glu Asp Arg Ile Val Trp Lys Thr Leu 945 950 955 960	2880

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ttc act gcc acg ata gag gag tgg caa gca gag cat gat gcc atc atg Phe Thr Ala Thr Ile Glu Glu Trp Gln Ala Glu His Asp Ala Ile Met 980 985 990	2976
agg cac atc ttg gag aga ccg gac cct acc gac gtc ttc cag aat aag Arg His Ile Leu Glu Arg Pro Asp Pro Thr Asp Val Phe Gln Asn Lys 995 1000 1005	3024
gca aac gtg tgt tgg gcc aag gct tta gtg ccg gtg ctg aag acc gct Ala Asn Val Cys Trp Ala Lys Ala Leu Val Pro Val Leu Lys Thr Ala 1010 1015 1020	3072
ggc ata gac atg acc act gaa caa tgg aac act gtg gat tat ttt gaa Gly Ile Asp Met Thr Thr Glu Gln Trp Asn Thr Val Asp Tyr Phe Glu 1025 1030 1035 1040	3120
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gtt ccg tta tcc att agg aat aat cac tgg gat aac tcc ccg tcg cct Val Pro Leu Ser Ile Arg Asn Asn His Trp Asp Asn Ser Pro Ser Pro 1075 1080 1085	3264
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tac cca caa ctg cct cgg gca gtt gcc act gga aga gtc tat gac atg Tyr Pro Gln Leu Pro Arg Ala Val Ala Thr Gly Arg Val Tyr Asp Met 1105 1110 1115 1120	3360
aac act ggt aca ctg cgc aat tat gat ccg cgc ata aac cta gta cct Asn Thr Gly Thr Leu Arg Asn Tyr Asp Pro Arg Ile Asn Leu Val Pro 1125 1130 1135	3408
gta aac aga aga ctg cct cat gct tta gtc ctc cac cat aat gaa cac Val Asn Arg Arg Leu Pro His Ala Leu Val Leu His His Asn Glu His 1140 1145 1150	3456
cca cag agt gac ttt tct tca ttc gtc agc aaa ttg aag ggc aga act Pro Gln Ser Asp Phe Ser Ser Phe Val Ser Lys Leu Lys Gly Arg Thr 1155 1160 1165	3504
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agg acc cca tat aaa tac cat cac tat cag cag tgt gaa gac cat gcc Arg Thr Pro Tyr Lys Tyr His His Tyr Gln Gln Cys Glu Asp His Ala 1220 1225 1230	3696
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tca acc ttg acc aac att tat aca ggt tcc aga ctc cac gaa gcc gga Ser Thr Leu Thr Asn Ile Tyr Thr Gly Ser Arg Leu His Glu Ala Gly 1315 1320 1325	3984
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gaa gga gtg att ata aat gct gct aac agc aaa gga caa cct ggc gga Glu Gly Val Ile Ile Asn Ala Ala Asn Ser Lys Gly Gln Pro Gly Gly 1345 1350 1355 1360	4080
ggg gtg tgc gga gcg ctg tat aag aag ttc ccg gaa agc ttc gat tta Gly Val Cys Gly Ala Leu Tyr Lys Lys Phe Pro Glu Ser Phe Asp Leu 1365 1370 1375	4128
cag ccg atc gaa gta gga aaa gcg cga ctg gtc aaa ggt gca gct aaa Gln Pro Ile Glu Val Gly Lys Ala Arg Leu Val Lys Gly Ala Ala Lys 1380 1385 1390	4176
cat atc att cat gcc gta gga cca aac ttc aac aaa gtt tcg gag gtt His Ile Ile His Ala Val Gly Pro Asn Phe Asn Lys Val Ser Glu Val 1395 1400 1405	4224

gaa ggt gac aaa cag ttg gca gag gct tat gag tcc atc gct aag att Glu Gly Asp Lys Gln Leu Ala Glu Ala Tyr Glu Ser Ile Ala Lys Ile 1410 1415 1420	4272
gtc aac gat aac aat tac aag tca gta gcg att cca ctg ttg tcc acc Val Asn Asp Asn Asn Tyr Lys Ser Val Ala Ile Pro Leu Leu Ser Thr 1425 1430 1435 1440	4320
ggc atc ttt tcc ggg aac aaa gat cga cta acc caa tca ttg aac cat Gly Ile Phe Ser Gly Asn Lys Asp Arg Leu Thr Gln Ser Leu Asn His 1445 1450 1455	4368
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gaa gca gtg gag gag ata tgc ata tcc gac gac tct tca gtg aca gaa Glu Ala Val Glu Glu Ile Cys Ile Ser Asp Asp Ser Ser Val Thr Glu 1490 1495 1500	4512
cct gat gca gag ctg gtg agg gtg cat ccg aag agt tct ttg gct gga Pro Asp Ala Glu Leu Val Arg Val His Pro Lys Ser Ser Leu Ala Gly 1505 1510 1515 1520	4560
agg aag ggc tac agc aca agc gat ggc aaa act ttc tca tat ttg gaa Arg Lys Gly Tyr Ser Thr Ser Asp Gly Lys Thr Phe Ser Tyr Leu Glu 1525 1530 1535	4608
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gcc atg act cca gaa aga gta cag cgc cta aaa gcc tca cgt cca gaa Ala Met Thr Pro Glu Arg Val Gln Arg Leu Lys Ala Ser Arg Pro Glu 1605 1610 1615	4848
caa att act gtg tgc tca tcc ttt cca ttg ccg aag tat aga atc act Gln Ile Thr Val Cys Ser Ser Phe Pro Leu Pro Lys Tyr Arg Ile Thr 1620 1625 1630	4896

ggt gtg cag aag atc caa tgc tcc cag cct ata ttg ttc tca ccg aaa Gly Val Gln Lys Ile Gln Cys Ser Gln Pro Ile Leu Phe Ser Pro Lys 1635 1640 1645	4944
gtg cct gcg tat att cat cca agg aag tat ctc gtg gaa aca cca ccg Val Pro Ala Tyr Ile His Pro Arg Lys Tyr Leu Val Glu Thr Pro Pro 1650 1655 1660	4992
gta gac gag act ccg gag cca tcg gca gag aac caa tcc aca gag ggg Val Asp Glu Thr Pro Glu Pro Ser Ala Glu Asn Gln Ser Thr Glu Gly 1665 1670 1675 1680	5040
aca cct gaa caa cca cca ctt ata acc gag gat, gag acc agg act aga Thr Pro Glu Gln Pro Pro Leu Ile Thr Glu Asp Glu Thr Arg Thr Arg 1685 1690 1695	5088
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ttg ctg tca gat ggc ccg acc cac cag gtg ctg caa gtc gag gca gac Leu Leu Ser Asp Gly Pro Thr His Gln Val Leu Gln Val Glu Ala Asp 1715 1720 1725	5184
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gca tcc gac ttt gat gtg gac agt tta tcc ata ctt gac acc ctg gag Ala Ser Asp Phe Asp Val Asp Ser Leu Ser Ile Leu Asp Thr Leu Glu 1745 1750 1755 1760	5280
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tca ctt gca ccc agc agg gcc tgc tcg aga acc agc cta gtt tcc acc Ser Leu Ala Pro Ser Arg Ala Cys Ser Arg Thr Ser Leu Val Ser Thr 1810 1815 1820	5472
ccg cca ggc gtg aat agg gtg atc act aga gag gag ctc gag gcg ctt Pro Pro Gly Val Asn Arg Val Ile Thr Arg Glu Glu Leu Glu Ala Leu 1825 1830 1835 1840	5520
acc ccg tca cgc act cct agc agg tcg gtc tcg aga acc agc ctg gtc Thr Pro Ser Arg Thr Pro Ser Arg Ser Val Ser Arg Thr Ser Leu Val 1845 1850 1855	5568

tcc aac ccg cca ggc gta aat agg gtg att aca aga gag gag ttt gag Ser Asn Pro Pro Gly Val Asn Arg Val Ile Thr Arg Glu Glu Phe Glu 1860 1865 1870	5616
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tta aaa gga cca aaa gct gct ctt ttt gcg aag aca cat aat ttg Leu Lys Gly Pro Lys Ala Ala Leu Phe Ala Lys Thr His Asn Leu 2145 2150 2155	6480
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act aaa ttt aaa ttc gga gcc atg atg aaa tct gga atg ttc ctc aca Thr Lys Phe Lys Phe Gly Ala Met Met Lys Ser Gly Met Phe Leu Thr	6960
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2320 2325 2330 2335	
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2340 2345 2350	
aat atc gtg aaa gga gtc aaa tcg gac aaa tta atg gca gac agg tgc Asn Ile Val Lys Gly Val Lys Ser Asp Lys Leu Met Ala Asp Arg Cys	7104
2355 2360 2365	
gcc acc tgg ttg aat atg gaa gtc aag att ata gat gct gtg gtg ggc Ala Thr Trp Leu Asn Met Glu Val Lys Ile Ile Asp Ala Val Val Gly	7152
2370 2375 2380	
gag aaa gcg ccc tat ttc tgt gga ggg ttt att ttg tgt gac tcc gtg Glu Lys Ala Pro Tyr Phe Cys Gly Gly Phe Ile Leu Cys Asp Ser Val	7200
2385 2390 2395	
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2400 2405 2410 2415	
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2420 2425 2430	
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2435 2440 2445	
tca gag ctg tgc aag gca gta gaa tca agg tat gaa acc gta gga act Ser Glu Leu Cys Lys Ala Val Glu Ser Arg Tyr Glu Thr Val Gly Thr	7392
2450 2455 2460	
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2465 2470 2475	
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2480 2485 2490	

<210> 3
<211> 2492
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 3

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 Ala Leu Gln Arg Ser Phe Pro Gln Phe Glu Val Glu Ala Lys Gln Val
 20 25 30
 Thr Asp Asn Asp His Ala Asn Ala Arg Ala Phe Ser His Leu Ala Ser
 35 40 45
 Lys Leu Ile Glu Thr Glu Val Asp Pro Ser Asp Thr Ile Leu Asp Ile
 50 55 60
 Gly Ser Ala Pro Ala Arg Arg Met Tyr Ser Lys His Lys Tyr His Cys
 65 70 75 80
 Ile Cys Pro Met Arg Cys Ala Glu Asp Pro Asp Arg Leu Tyr Lys Tyr
 85 90 95
 Ala Thr Lys Leu Lys Lys Asn Cys Lys Glu Ile Thr Asp Lys Glu Leu
 100 105 110
 Asp Lys Lys Met Lys Glu Leu Ala Ala Val Met Ser Asp Pro Asp Leu
 115 120 125
 Glu Thr Glu Thr Met Cys Leu His Asp Asp Glu Ser Cys Arg Tyr Glu
 130 135 140
 Gly Gln Val Ala Val Tyr Gln Asp Val Tyr Ala Val Asp Gly Pro Thr
 145 150 155 160
 Ser Leu Tyr His Gln Ala Asn Lys Gly Val Arg Val Ala Tyr Trp Ile
 165 170 175
 Gly Phe Asp Thr Thr Pro Phe Met Phe Lys Asn Leu Ala Gly Ala Tyr
 180 185 190
 Pro Ser Tyr Ser Thr Asn Trp Ala Asp Glu Thr Val Leu Thr Ala Arg
 195 200 205
 Asn Ile Gly Leu Cys Ser Ser Asp Val Met Glu Arg Ser Arg Arg Gly
 210 215 220
 Met Ser Ile Leu Arg Lys Lys Tyr Leu Lys Pro Ser Asn Asn Val Leu
 225 230 235 240
 Phe Ser Val Gly Ser Thr Ile Tyr His Glu Lys Arg Asp Leu Leu Arg
 245 250 255
 Ser Trp His Leu Pro Ser Val Phe His Leu Arg Gly Lys Gln Asn Tyr
 260 265 270
 Thr Cys Arg Cys Glu Thr Ile Val Ser Cys Asp Gly Tyr Val Val Lys
 275 280 285
 Arg Ile Ala Ile Ser Pro Gly Leu Tyr Gly Lys Pro Ser Gly Tyr Ala
 290 295 300
 Ala Thr Met His Arg Glu Gly Phe Leu Cys Cys Lys Val Thr Asp Thr
 305 310 315 320
 Leu Asn Gly Glu Arg Val Ser Phe Pro Val Cys Thr Tyr Val Pro Ala
 325 330 335
 Thr Leu Cys Asp Gln Met Thr Gly Ile Leu Ala Thr Asp Val Ser Ala
 340 345 350
 Asp Asp Ala Gln Lys Leu Leu Val Gly Leu Asn Gln Arg Ile Val Val
 355 360 365
 Asn Gly Arg Thr Gln Arg Asn Thr Asn Thr Met Lys Asn Tyr Leu Leu
 370 375 380
 Pro Val Val Ala Gln Ala Phe Ala Arg Trp Ala Lys Glu Tyr Lys Glu
 385 390 395 400

Asp Gln Glu Asp Glu Arg Pro Leu Gly Leu Arg Asp Arg Gln Leu Val
 405 410 415
 Met Gly Cys Cys Trp Ala Phe Arg Arg His Lys Ile Thr Ser Ile Tyr
 420 425 430
 Lys Arg Pro Asp Thr Gln Thr Ile Ile Lys Val Asn Ser Asp Phe His
 435 440 445
 Ser Phe Val Leu Pro Arg Ile Gly Ser Asn Thr Leu Glu Ile Gly Leu
 450 455 460
 Arg Thr Arg Ile Arg Lys Met Leu Glu Glu His Lys Glu Pro Ser Pro
 465 470 475 480
 Leu Ile Thr Ala Glu Asp Val Gln Glu Ala Lys Cys Ala Ala Asp Glu
 485 490 495
 Ala Lys Glu Val Arg Glu Ala Glu Glu Leu Arg Ala Ala Leu Pro Pro
 500 505 510
 Leu Ala Ala Asp Val Glu Glu Pro Thr Leu Glu Ala Asp Val Asp Leu
 515 520 525
 Met Leu Gln Glu Ala Gly Ala Gly Ser Val Glu Thr Pro Arg Gly Leu
 530 535 540
 Ile Lys Val Thr Ser Tyr Ala Gly Glu Asp Lys Ile Gly Ser Tyr Ala
 545 550 555 560
 Val Leu Ser Pro Gln Ala Val Leu Lys Ser Glu Lys Leu Ser Cys Ile
 565 570 575
 His Pro Leu Ala Glu Gln Val Ile Val Ile Thr His Ser Gly Arg Lys
 580 585 590
 Gly Arg Tyr Ala Val Glu Pro Tyr His Gly Lys Val Val Val Pro Glu
 595 600 605
 Gly His Ala Ile Pro Val Gln Asp Phe Gln Ala Leu Ser Glu Ser Ala
 610 615 620
 Thr Ile Val Tyr Asn Glu Arg Glu Phe Val Asn Arg Tyr Leu His His
 625 630 635 640
 Ile Ala Thr His Gly Gly Ala Leu Asn Thr Asp Glu Glu Tyr Tyr Lys
 645 650 655
 Thr Val Lys Pro Ser Glu His Asp Gly Glu Tyr Leu Tyr Asp Ile Asp
 660 665 670
 Arg Lys Gln Cys Val Lys Lys Glu Leu Val Thr Gly Leu Gly Leu Thr
 675 680 685
 Gly Glu Leu Val Asp Pro Pro Phe His Glu Phe Ala Tyr Glu Ser Leu
 690 695 700
 Arg Thr Arg Pro Ala Ala Pro Tyr Gln Val Pro Thr Ile Gly Val Tyr
 705 710 715 720
 Gly Val Pro Gly Ser Gly Lys Ser Gly Ile Ile Lys Ser Ala Val Thr
 725 730 735
 Lys Lys Asp Leu Val Val Ser Ala Lys Lys Glu Asn Cys Ala Glu Ile
 740 745 750
 Ile Arg Asp Val Lys Lys Met Lys Gly Leu Asp Val Asn Ala Arg Thr
 755 760 765
 Val Asp Ser Val Leu Leu Asn Gly Cys Lys His Pro Val Glu Thr Leu
 770 775 780
 Tyr Ile Asp Glu Ala Phe Ala Cys His Ala Gly Thr Leu Arg Ala Leu
 785 790 795 800
 Ile Ala Ile Ile Arg Pro Lys Lys Ala Val Leu Cys Gly Asp Pro Lys
 805 810 815
 Gln Cys Gly Phe Phe Asn Met Met Cys Leu Lys Val His Phe Asn His
 820 825 830

Glu Ile Cys Thr Gln Val Phe His Lys Ser Ile Ser Arg Arg Cys Thr
 835 840 845
 Lys Ser Val Thr Ser Val Val Ser Thr Leu Phe Tyr Asp Lys Lys Met
 850 855 860
 Arg Thr Thr Asn Pro Lys Glu Thr Lys Ile Val Ile Asp Thr Thr Gly
 865 870 875 880
 Ser Thr Lys Pro Lys Gln Asp Asp Leu Ile Leu Thr Cys Phe Arg Gly
 885 890 895
 Trp Val Lys Gln Leu Gln Ile Asp Tyr Lys Gly Asn Glu Ile Met Thr
 900 905 910
 Ala Ala Ala Ser Gln Gly Leu Thr Arg Lys Gly Val Tyr Ala Val Arg
 915 920 925
 Tyr Lys Val Asn Glu Asn Pro Leu Tyr Ala Pro Thr Ser Glu His Val
 930 935 940
 Asn Val Leu Leu Thr Arg Thr Glu Asp Arg Ile Val Trp Lys Thr Leu
 945 950 955 960
 Ala Gly Asp Pro Trp Ile Lys Thr Leu Thr Ala Lys Tyr Pro Gly Asn
 965 970 975
 Phe Thr Ala Thr Ile Glu Glu Trp Gln Ala Glu His Asp Ala Ile Met
 980 985 990
 Arg His Ile Leu Glu Arg Pro Asp Pro Thr Asp Val Phe Gln Asn Lys
 995 1000 1005
 Ala Asn Val Cys Trp Ala Lys Ala Leu Val Pro Val Leu Lys Thr Ala
 1010 1015 1020
 Gly Ile Asp Met Thr Thr Glu Gln Trp Asn Thr Val Asp Tyr Phe Glu
 1025 1030 1035 1040
 Thr Asp Lys Ala His Ser Ala Glu Ile Val Leu Asn Gln Leu Cys Val
 1045 1050 1055
 Arg Phe Phe Gly Leu Asp Leu Asp Ser Gly Leu Phe Ser Ala Pro Thr
 1060 1065 1070
 Val Pro Leu Ser Ile Arg Asn Asn His Trp Asp Asn Ser Pro Ser Pro
 1075 1080 1085
 Asn Met Tyr Gly Leu Asn Lys Glu Val Val Arg Gln Leu Ser Arg Arg
 1090 1095 1100
 Tyr Pro Gln Leu Pro Arg Ala Val Ala Thr Gly Arg Val Tyr Asp Met
 1105 1110 1115 1120
 Asn Thr Gly Thr Leu Arg Asn Tyr Asp Pro Arg Ile Asn Leu Val Pro
 1125 1130 1135
 Val Asn Arg Arg Leu Pro His Ala Leu Val Leu His His Asn Glu His
 1140 1145 1150
 Pro Gln Ser Asp Phe Ser Ser Phe Val Ser Lys Leu Lys Gly Arg Thr
 1155 1160 1165
 Val Leu Val Val Gly Glu Lys Leu Ser Val Pro Gly Lys Met Val Asp
 1170 1175 1180
 Trp Leu Ser Asp Arg Pro Glu Ala Thr Phe Arg Ala Arg Leu Asp Leu
 1185 1190 1195 1200
 Gly Ile Pro Gly Asp Val Pro Lys Tyr Asp Ile Ile Phe Val Asn Val
 1205 1210 1215
 Arg Thr Pro Tyr Lys Tyr His His Tyr Gln Gln Cys Glu Asp His Ala
 1220 1225 1230
 Ile Lys Leu Ser Met Leu Thr Lys Lys Ala Cys Leu His Leu Asn Pro
 1235 1240 1245
 Gly Gly Thr Cys Val Ser Ile Gly Tyr Gly Tyr Ala Asp Arg Ala Ser
 1250 1255 1260

Glu Ser Ile Ile Gly Ala Ile Ala Arg Gln Phe Lys Phe Ser Arg Val
 1265 1270 1275 1280
 Cys Lys Pro Lys Ser Ser Leu Glu Glu Thr Glu Val Leu Phe Val Phe
 1285 1290 1295
 Ile Gly Tyr Asp Arg Lys Ala Arg Thr His Asn Pro Tyr Lys Leu Ser
 1300 1305 1310
 Ser Thr Leu Thr Asn Ile Tyr Thr Gly Ser Arg Leu His Glu Ala Gly
 1315 1320 1325
 Cys Ala Pro Ser Tyr His Val Val Arg Gly Asp Ile Ala Thr Ala Thr
 1330 1335 1340
 Glu Gly Val Ile Ile Asn Ala Ala Asn Ser Lys Gly Gln Pro Gly Gly
 1345 1350 1355 1360
 Gly Val Cys Gly Ala Leu Tyr Lys Lys Phe Pro Glu Ser Phe Asp Leu
 1365 1370 1375
 Gln Pro Ile Glu Val Gly Lys Ala Arg Leu Val Lys Gly Ala Ala Lys
 1380 1385 1390
 His Ile Ile His Ala Val Gly Pro Asn Phe Asn Lys Val Ser Glu Val
 1395 1400 1405
 Glu Gly Asp Lys Gln Leu Ala Glu Ala Tyr Glu Ser Ile Ala Lys Ile
 1410 1415 1420
 Val Asn Asp Asn Asn Tyr Lys Ser Val Ala Ile Pro Leu Leu Ser Thr
 1425 1430 1435 1440
 Gly Ile Phe Ser Gly Asn Lys Asp Arg Leu Thr Gln Ser Leu Asn His
 1445 1450 1455
 Leu Leu Thr Ala Leu Asp Thr Thr Asp Ala Asp Val Ala Ile Tyr Cys
 1460 1465 1470
 Arg Asp Lys Lys Trp Glu Met Thr Leu Lys Glu Ala Val Ala Arg Arg
 1475 1480 1485
 Glu Ala Val Glu Glu Ile Cys Ile Ser Asp Asp Ser Ser Val Thr Glu
 1490 1495 1500
 Pro Asp Ala Glu Leu Val Arg Val His Pro Lys Ser Ser Leu Ala Gly
 1505 1510 1515 1520
 Arg Lys Gly Tyr Ser Thr Ser Asp Gly Lys Thr Phe Ser Tyr Leu Glu
 1525 1530 1535
 Gly Thr Lys Phe His Gln Ala Ala Lys Asp Ile Ala Glu Ile Asn Ala
 1540 1545 1550
 Met Trp Pro Val Ala Thr Glu Ala Asn Glu Gln Val Cys Met Tyr Ile
 1555 1560 1565
 Leu Gly Glu Ser Met Ser Ser Ile Arg Ser Lys Cys Pro Val Glu Glu
 1570 1575 1580
 Ser Glu Ala Ser Thr Pro Pro Ser Thr Leu Pro Cys Leu Cys Ile His
 1585 1590 1595 1600
 Ala Met Thr Pro Glu Arg Val Gln Arg Leu Lys Ala Ser Arg Pro Glu
 1605 1610 1615
 Gln Ile Thr Val Cys Ser Ser Phe Pro Leu Pro Lys Tyr Arg Ile Thr
 1620 1625 1630
 Gly Val Gln Lys Ile Gln Cys Ser Gln Pro Ile Leu Phe Ser Pro Lys
 1635 1640 1645
 Val Pro Ala Tyr Ile His Pro Arg Lys Tyr Leu Val Glu Thr Pro Pro
 1650 1655 1660
 Val Asp Glu Thr Pro Glu Pro Ser Ala Glu Asn Gln Ser Thr Glu Gly
 1665 1670 1675 1680
 Thr Pro Glu Gln Pro Pro Leu Ile Thr Glu Asp Glu Thr Arg Thr Arg
 1685 1690 1695

Thr Pro Glu Pro Ile Ile Ile Glu Glu Glu Glu Asp Ser Ile Ser
 1700 1705 1710
 Leu Leu Ser Asp Gly Pro Thr His Gln Val Leu Gln Val Glu Ala Asp
 1715 1720 1725
 Ile His Gly Pro Pro Ser Val Ser Ser Ser Trp Ser Ile Pro His
 1730 1735 1740
 Ala Ser Asp Phe Asp Val Asp Ser Leu Ser Ile Leu Asp Thr Leu Glu
 1745 1750 1755 1760
 Gly Ala Ser Val Thr Ser Gly Ala Thr Ser Ala Glu Thr Asn Ser Tyr
 1765 1770 1775
 Phe Ala Lys Ser Met Glu Phe Leu Ala Arg Pro Val Pro Ala Pro Arg
 1780 1785 1790
 Thr Val Phe Arg Asn Pro Pro His Pro Ala Pro Arg Thr Arg Thr Pro
 1795 1800 1805
 Ser Leu Ala Pro Ser Arg Ala Cys Ser Arg Thr Ser Leu Val Ser Thr
 1810 1815 1820
 Pro Pro Gly Val Asn Arg Val Ile Thr Arg Glu Glu Leu Glu Ala Leu
 1825 1830 1835 1840
 Thr Pro Ser Arg Thr Pro Ser Arg Ser Val Ser Arg Thr Ser Leu Val
 1845 1850 1855
 Ser Asn Pro Pro Gly Val Asn Arg Val Ile Thr Arg Glu Glu Phe Glu
 1860 1865 1870
 Ala Phe Val Ala Gln Gln Gln Arg Phe Asp Ala Gly Ala Tyr Ile Phe
 1875 1880 1885
 Ser Ser Asp Thr Gly Gln Gly His Leu Gln Gln Lys Ser Val Arg Gln
 1890 1895 1900
 Thr Val Leu Ser Glu Val Val Leu Glu Arg Thr Glu Leu Glu Ile Ser
 1905 1910 1915 1920
 Tyr Ala Pro Arg Leu Asp Gln Glu Lys Glu Glu Leu Leu Arg Lys Lys
 1925 1930 1935
 Leu Gln Leu Asn Pro Thr Pro Ala Asn Arg Ser Arg Tyr Gln Ser Arg
 1940 1945 1950
 Lys Val Glu Asn Met Lys Ala Ile Thr Ala Arg Arg Ile Leu Gln Gly
 1955 1960 1965
 Leu Gly His Tyr Leu Lys Ala Glu Gly Lys Val Glu Cys Tyr Arg Thr
 1970 1975 1980
 Leu His Pro Val Pro Leu Tyr Ser Ser Val Asn Arg Ala Phe Ser
 1985 1990 1995 2000
 Ser Pro Lys Val Ala Val Glu Ala Cys Asn Ala Met Leu Lys Glu Asn
 2005 2010 2015
 Phe Pro Thr Val Ala Ser Tyr Cys Ile Ile Pro Glu Tyr Asp Ala Tyr
 2020 2025 2030
 Leu Asp Met Val Asp Gly Ala Ser Cys Cys Leu Asp Thr Ala Ser Phe
 2035 2040 2045
 Cys Pro Ala Lys Leu Arg Ser Phe Pro Lys Lys His Ser Tyr Leu Glu
 2050 2055 2060
 Pro Thr Ile Arg Ser Ala Val Pro Ser Ala Ile Gln Asn Thr Leu Gln
 2065 2070 2075 2080
 Asn Val Leu Ala Ala Ala Thr Lys Arg Asn Cys Asn Val Thr Gln Met
 2085 2090 2095
 Arg Glu Leu Pro Val Leu Asp Ser Ala Ala Phe Asn Val Glu Cys Phe
 2100 2105 2110
 Lys Lys Tyr Ala Cys Asn Asn Glu Tyr Trp Glu Thr Phe Lys Glu Asn
 2115 2120 2125

Pro Ile Arg Leu Thr Glu Glu Asn Val Val Asn Tyr Ile Thr Lys Leu
 2130 2135 2140
 Lys Gly Pro Lys Ala Ala Ala Leu Phe Ala Lys Thr His Asn Leu Asn
 2145 2150 2155 2160
 Met Leu Gln Asp Ile Pro Met Asp Arg Phe Val Met Asp Leu Lys Arg
 2165 2170 2175
 Asp Val Lys Val Thr Pro Gly Thr Lys His Thr Glu Glu Arg Pro Lys
 2180 2185 2190
 Val Gln Val Ile Gln Ala Ala Asp Pro Leu Ala Thr Ala Tyr Leu Cys
 2195 2200 2205
 Gly Ile His Arg Glu Leu Val Arg Arg Leu Asn Ala Val Leu Leu Pro
 2210 2215 2220
 Asn Ile His Thr Leu Phe Asp Met Ser Ala Glu Asp Phe Asp Ala Ile
 2225 2230 2235 2240
 Ile Ala Glu His Phe Gln Pro Gly Asp Cys Val Leu Glu Thr Asp Ile
 2245 2250 2255
 Ala Ser Phe Asp Lys Ser Glu Asp Asp Ala Met Ala Leu Thr Ala Leu
 2260 2265 2270
 Met Ile Leu Glu Asp Leu Gly Val Asp Ala Glu Leu Leu Thr Leu Ile
 2275 2280 2285
 Glu Ala Ala Phe Gly Glu Ile Ser Ser Ile His Leu Pro Thr Lys Thr
 2290 2295 2300
 Lys Phe Lys Phe Gly Ala Met Met Lys Ser Gly Met Phe Leu Thr Leu
 2305 2310 2315 2320
 Phe Val Asn Thr Val Ile Asn Ile Val Ile Ala Ser Arg Val Leu Arg
 2325 2330 2335
 Glu Arg Leu Thr Gly Ser Pro Cys Ala Ala Phe Ile Gly Asp Asp Asn
 2340 2345 2350
 Ile Val Lys Gly Val Lys Ser Asp Lys Leu Met Ala Asp Arg Cys Ala
 2355 2360 2365
 Thr Trp Leu Asn Met Glu Val Lys Ile Ile Asp Ala Val Val Gly Glu
 2370 2375 2380
 Lys Ala Pro Tyr Phe Cys Gly Gly Phe Ile Leu Cys Asp Ser Val Thr
 2385 2390 2395 2400
 Gly Thr Ala Cys Arg Val Ala Asp Pro Leu Lys Arg Leu Phe Lys Leu
 2405 2410 2415
 Gly Lys Pro Leu Ala Ala Asp Asp Glu His Asp Asp Asp Arg Arg Arg
 2420 2425 2430
 Ala Leu His Glu Glu Ser Thr Arg Trp Asn Arg Val Gly Ile Leu Ser
 2435 2440 2445
 Glu Leu Cys Lys Ala Val Glu Ser Arg Tyr Glu Thr Val Gly Thr Ser
 2450 2455 2460
 Ile Ile Val Met Ala Met Thr Thr Leu Ala Ser Ser Val Lys Ser Phe
 2465 2470 2475 2480
 Ser Tyr Leu Arg Gly Ala Pro Ile Thr Leu Tyr Gly
 2485 2490

<210> 4
 <211> 1476
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence; Note =
 synthetic construct

<221> CDS
<222> (1)...(1476)

<400> 4

atg gct gcg aga gca tca ata tta aga ggg gaa aaa tta gat aaa tgg	48
Met Ala Ala Arg Ala Ser Ile Leu Arg Gly Glu Lys Leu Asp Lys Trp	
1 5 10 15	
gaa aag att agg tta agg cca ggg gga aag aaa cat tat atg tta aaa	96
Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys His Tyr Met Leu Lys	
20 25 30	
cac ata gta tgg gcg agc agg gag ctg gaa aga ttt gca ctt aac cct	144
His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Leu Asn Pro	
35 40 45	
ggc ctt tta gaa aca tca gaa gga tgt aaa caa ata atg aaa cag cta	192
Gly Leu Leu Glu Thr Ser Glu Gly Cys Lys Gln Ile Met Lys Gln Leu	
50 55 60	
caa cca gct ctc cag aca gga aca gag gaa ctt aaa tca tta tac aac	240
Gln Pro Ala Leu Gln Thr Gly Thr Glu Glu Leu Lys Ser Leu Tyr Asn	
65 70 75 80	
aca gta gca act ctc tat tgt gta cat gaa aag ata gaa gta cga gac	288
Thr Val Ala Thr Leu Tyr Cys Val His Glu Lys Ile Glu Val Arg Asp	
85 90 95	
acc aag gaa gcc tta gat aag ata gag gaa gaa caa aac aaa tgt cag	336
Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Cys Gln	
100 105 110	
caa aaa acg cag cag gca aaa gcg gct gac ggg aaa gtc agt caa aat	384
Gln Lys Thr Gln Gln Ala Lys Ala Ala Asp Gly Lys Val Ser Gln Asn	
115 120 125	
tat cct ata gtg cag aat ctc caa ggg caa atg gta cat caa gcc ata	432
Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile	
130 135 140	
tca cct aga acc ttg aat gca tgg gta aaa gta ata gaa gaa aag gct	480
Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala	
145 150 155 160	
ttt agc cca gag gta ata ccc atg ttt aca gca tta tca gaa gga gcc	528
Phe Ser Pro Glu Val Ile Pro Met Phe Thr Ala Leu Ser Glu Gly Ala	
165 170 175	
acc cca caa gat tta aac acc atg tta aat aca gtg ggg gga cac caa	576
Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln	
180 185 190	
gca gcc atg caa atg tta aaa gat act att aat gaa gag gct gca gaa	624
Ala Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu	
195 200 205	

tgg gat aga tta cat cca gtc cat gcg ggg cct att gca cca ggc cag Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln 210 215 220	672
atg aga gaa cca agg gga agt gac ata gca gga act act agt acc ctt Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu 225 230 235 240	720
cag gaa caa ata gca tgg atg aca agt aac cca cct att cca gtg gga Gln Glu Gln Ile Ala Trp Met Thr Ser Asn Pro Pro Ile Pro Val Gly 245 250 255	768
gac atc tat aaa aga tgg ata att ctg ggg tta aat aaa ata gtg aga. Asp Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg 260 265 270	816
atg tat agc ccg gtc agc att ttg gac ata aga caa ggg cca aag gaa Met Tyr Ser Pro Val Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu 275 280 285	864
ccc ttt cga gac tat gta gat cgg ttc ttt aaa act tta aga gct gaa Pro Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala Glu 290 295 300	912
caa gct aca caa gaa gta aaa aat tgg atg aca gac acc ttg tta gtc Gln Ala Thr Gln Glu Val Lys Asn Trp Met Thr Asp Thr Leu Leu Val 305 310 315 320	960
caa aat gcg aac cca gat tgt aag acc att ttg aga gca tta gga cca Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Arg Ala Leu Gly Pro 325 330 335	1008
ggg gct aca tta gaa gaa atg atg aca gca tgt caa ggg gtg gga gga Gly Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly 340 345 350	1056
cct ggc cac aaa gca aga gta ttg gct gag gca atg agt caa aca aac Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Thr Asn 355 360 365	1104
agt gga aac ata atg atg cag aga agc aat ttt aaa ggc cct aga aga Ser Gly Asn Ile Met Met Gln Arg Ser Asn Phe Lys Gly Pro Arg Arg 370 375 380	1152
att gtt aaa tgt ttt aac tgt ggc aag gaa ggg cac ata gcc aga aat Ile Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn 385 390 395 400	1200
tgc aga gcc cct agg aaa aaa ggc tgt tgg aaa tgt gga aaa gaa gga Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly 405 410 415	1248
cac caa atg aaa gac tgc act gag agg cag gct aat ttt tta ggg aaa His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys 420 425 430	1296

att tgg cct tcc cac aag ggg agg cca ggg aat ttc ctt cag aac aga		1344
Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Asn Arg		
435	440	445
cca gag cca aca gcc cca cca gca gag agc ttc aqg ttc gaa gag aca		1392
Pro Glu Pro Thr Ala Pro Pro Ala Glu Ser Phe Arg Phe Glu Glu Thr		
450	455	460
acc ccc gct ccg aaa cag gag ccg ata gaa agg gaa ccc tta act tcc		1440
Thr Pro Ala Pro Lys Gln Glu Pro Ile Glu Arg Glu Pro Leu Thr Ser		
465	470	475
ctc aaa tca ctc ttt ggc agc gac ccc ttg tct caa		1476
Leu Lys Ser Leu Phe Gly Ser Asp Pro Leu Ser Gln		
485	490	

<210> 5
<211> 492
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 5			
Met Ala Ala Arg Ala Ser Ile Leu Arg Gly Glu Lys Leu Asp Lys Trp			
1	5	10	15
Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys His Tyr Met Leu Lys			
20	25	30	
His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Leu Asn Pro			
35	40	45	
Gly Leu Leu Glu Thr Ser Glu Gly Cys Lys Gln Ile Met Lys Gln Leu			
50	55	60	
Gln Pro Ala Leu Gln Thr Gly Thr Glu Glu Leu Lys Ser Leu Tyr Asn			
65	70	75	80
Thr Val Ala Thr Leu Tyr Cys Val His Glu Lys Ile Glu Val Arg Asp			
85	90	95	
Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Cys Gln			
100	105	110	
Gln Lys Thr Gln Gln Ala Lys Ala Ala Asp Gly Lys Val Ser Gln Asn			
115	120	125	
Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile			
130	135	140	
Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala			
145	150	155	160
Phe Ser Pro Glu Val Ile Pro Met Phe Thr Ala Leu Ser Glu Gly Ala			
165	170	175	
Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln			
180	185	190	
Ala Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu			
195	200	205	

Trp	Asp	Arg	Leu	His	Pro	Val	His	Ala	Gly	Pro	Ile	Ala	Pro	Gly	Gln
210					215						220				
Met	Arg	Glu	Pro	Arg	Gly	Ser	Asp	Ile	Ala	Gly	Thr	Thr	Ser	Thr	Leu
225					230						235				240
Gln	Glu	Gln	Ile	Ala	Trp	Met	Thr	Ser	Asn	Pro	Pro	Ile	Pro	Val	Gly
					245					250				255	
Asp	Ile	Tyr	Lys	Arg	Trp	Ile	Ile	Leu	Gly	Leu	Asn	Lys	Ile	Val	Arg
					260				265				270		
Met	Tyr	Ser	Pro	Val	Ser	Ile	Leu	Asp	Ile	Arg	Gln	Gly	Pro	Lys	Glu
					275				280			285			
Pro	Phe	Arg	Asp	Tyr	Val	Asp	Arg	Phe	Phe	Lys	Thr	Leu	Arg	Ala	Glu
					290			295			300				
Gln	Ala	Thr	Gln	Glu	Val	Lys	Asn	Trp	Met	Thr	Asp	Thr	Leu	Leu	Val
305					310					315				320	
Gln	Asn	Ala	Asn	Pro	Asp	Cys	Lys	Thr	Ile	Leu	Arg	Ala	Leu	Gly	Pro
					325				330				335		
Gly	Ala	Thr	Leu	Glu	Glu	Met	Met	Thr	Ala	Cys	Gln	Gly	Val	Gly	Gly
					340				345			350			
Pro	Gly	His	Lys	Ala	Arg	Val	Leu	Ala	Glu	Ala	Met	Ser	Gln	Thr	Asn
					355				360			365			
Ser	Gly	Asn	Ile	Met	Met	Gln	Arg	Ser	Asn	Phe	Lys	Gly	Pro	Arg	Arg
					370			375			380				
Ile	Val	Lys	Cys	Phe	Asn	Cys	Gly	Lys	Glu	Gly	His	Ile	Ala	Arg	Asn
385					390				395				400		
Cys	Arg	Ala	Pro	Arg	Lys	Lys	Gly	Cys	Trp	Lys	Cys	Gly	Lys	Glu	Gly
					405				410			415			
His	Gln	Met	Lys	Asp	Cys	Thr	Glu	Arg	Gln	Ala	Asn	Phe	Leu	Gly	Lys
					420				425			430			
Ile	Trp	Pro	Ser	His	Lys	Gly	Arg	Pro	Gly	Asn	Phe	Leu	Gln	Asn	Arg
					435			440			445				
Pro	Glu	Pro	Thr	Ala	Pro	Pro	Ala	Glu	Ser	Phe	Arg	Phe	Glu	Glu	Thr
					450			455			460				
Thr	Pro	Ala	Pro	Lys	Gln	Glu	Pro	Ile	Glu	Arg	Glu	Pro	Leu	Thr	Ser
465					470				475				480		
Leu	Lys	Ser	Leu	Phe	Gly	Ser	Asp	Pro	Leu	Ser	Gln				
					485				490						

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<210> 6
<211> 813
<212> DNA
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> CDS
<222> (1) ... (813)

<400> 6
atg agc cat att caa cg^g gaa ac^g tct tgc tc^g agg cc^g cga tt^a aat
Met Ser His Ile Gln Arg Glu Thr Ser Cys Ser Arg Pro Arg Leu Asn
1 5 10 15

tcc aac atg gat gct gat tta tat ggg tat aaa tgg gct cgc gat aat Ser Asn Met Asp Ala Asp Leu Tyr Gly Tyr Lys Trp Ala Arg Asp Asn	96
20 25 30	
gtc ggg caa tca ggt gcg aca atc tat cga ttg tat ggg aag ccc gat Val Gly Gln Ser Gly Ala Thr Ile Tyr Arg Leu Tyr Gly Lys Pro Asp	144
35 40 45	
gcg cca gag ttg ttt ctg aaa cat ggc aaa ggt agc gtt gcc aat gat Ala Pro Glu Leu Phe Leu Lys His Gly Lys Ser Val Ala Asn Asp	192
50 55 60	
gtt aca gat gag atg gtc aga cta aac tgg ctg acg gaa ttt atg cct Val Thr Asp Glu Met Val Arg Leu Asn Trp Leu Thr Glu Phe Met Pro	240
65 70 75 80	
ctt ccg acc atc aag cat ttt atc cgt act cct gat gat gca tgg tta Leu Pro Thr Ile Lys His Phe Ile Arg Thr Pro Asp Asp Ala Trp Leu	288
85 90 95	
ctc acc act gcg atc ccc ggg aaa aca gca ttc cag gta tta gaa gaa Leu Thr Thr Ala Ile Pro Gly Lys Thr Ala Phe Gln Val Leu Glu Glu	336
100 105 110	
tat cct gat tca ggt gaa aat att gtt gat gcg ctg gca gtg ttc ctg Tyr Pro Asp Ser Gly Glu Asn Ile Val Asp Ala Leu Ala Val Phe Leu	384
115 120 125	
cgc cgg ttg cat tcg att cct gtt tgt aat tgt cct ttt aac agc gat Arg Arg Leu His Ser Ile Pro Val Cys Asn Cys Pro Phe Asn Ser Asp	432
130 135 140	
cgc gta ttt cgt ctc gct cag gcg caa tca cga atg aat aac ggt ttg Arg Val Phe Arg Leu Ala Gln Ala Gln Ser Arg Met Asn Asn Gly Leu	480
145 150 155 160	
gtt gat gcg agt gat ttt gat gac gag cgt aat ggc tgg cct gtt gaa Val Asp Ala Ser Asp Phe Asp Asp Glu Arg Asn Gly Trp Pro Val Glu	528
165 170 175	
caa gtc tgg aaa gaa atg cat aag ctt ttg cca ttc tca ccg gat tca Gln Val Trp Lys Glu Met His Lys Leu Leu Pro Phe Ser Pro Asp Ser	576
180 185 190	
gtc gtc act cat ggt gat ttc tca ctt gat aac ctt att ttt gac gag Val Val Thr His Gly Asp Phe Ser Leu Asp Asn Leu Ile Phe Asp Glu	624
195 200 205	
ggg aaa tta ata ggt tgt att gat gtt gga cga gtc gga atc gca gac Gly Lys Leu Ile Gly Cys Ile Asp Val Gly Arg Val Gly Ile Ala Asp	672
210 215 220	
cga tac cag gat ctt gcc atc cta tgg aac tgc ctc ggt gag ttt tct Arg Tyr Gln Asp Leu Ala Ile Leu Trp Asn Cys Leu Gly Glu Phe Ser	720
225 230 235 240	

cct tca tta cag aaa cg^g ctt ttt caa aaa tat gg^t att gat aat cct 768
 Pro Ser Leu Gln Lys Arg Leu Phe Gln Lys Tyr Gly Ile Asp Asn Pro
 245 250 255

gat atg aat aaa ttg cag ttt cat ttg atg ctc gat gag ttt ttc 813
 Asp Met Asn Lys Leu Gln Phe His Leu Met Leu Asp Glu Phe Phe
 260 265 270

<210> 7
<211> 271
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 7
 Met Ser His Ile Gln Arg Glu Thr Ser Cys Ser Arg Pro Arg Leu Asn
 1 5 10 15
 Ser Asn Met Asp Ala Asp Leu Tyr Gly Tyr Lys Trp Ala Arg Asp Asn
 20 25 30
 Val Gly Gln Ser Gly Ala Thr Ile Tyr Arg Leu Tyr Gly Lys Pro Asp
 35 40 45
 Ala Pro Glu Leu Phe Leu Lys His Gly Lys Gly Ser Val Ala Asn Asp
 50 55 60
 Val Thr Asp Glu Met Val Arg Leu Asn Trp Leu Thr Glu Phe Met Pro
 65 70 75 80
 Leu Pro Thr Ile Lys His Phe Ile Arg Thr Pro Asp Asp Ala Trp Leu
 85 90 95
 Leu Thr Thr Ala Ile Pro Gly Lys Thr Ala Phe Gln Val Leu Glu Glu
 100 105 110
 Tyr Pro Asp Ser Gly Glu Asn Ile Val Asp Ala Leu Ala Val Phe Leu
 115 120 125
 Arg Arg Leu His Ser Ile Pro Val Cys Asn Cys Pro Phe Asn Ser Asp
 130 135 140
 Arg Val Phe Arg Leu Ala Gln Ala Gln Ser Arg Met Asn Asn Gly Leu
 145 150 155 160
 Val Asp Ala Ser Asp Phe Asp Asp Glu Arg Asn Gly Trp Pro Val Glu
 165 170 175
 Gin Val Trp Lys Glu Met His Lys Leu Leu Pro Phe Ser Pro Asp Ser
 180 185 190
 Val Val Thr His Gly Asp Phe Ser Leu Asp Asn Leu Ile Phe Asp Glu
 195 200 205
 Gly Lys Leu Ile Gly Cys Ile Asp Val Gly Arg Val Gly Ile Ala Asp
 210 215 220
 Arg Tyr Gln Asp Leu Ala Ile Leu Trp Asn Cys Leu Gly Glu Phe Ser
 225 230 235 240
 Pro Ser Leu Gln Lys Arg Leu Phe Gln Lys Tyr Gly Ile Asp Asn Pro
 245 250 255
 Asp Met Asn Lys Leu Gln Phe His Leu Met Leu Asp Glu Phe Phe
 260 265 270

<210> 8
<211> 5076
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

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tggcttcaaa actgatcgaa acggagggtgg accccatccga cacgatcctt gacattggaa	240
gtgcgcggc cccgagaatg tattctaagc acaagtatca ttgtatctgt ccgatgagat	300
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cgatgcagcc aatgcctat cgcaacccgt tgcggcccc ggcgaggccc tggttccccaa	660
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aagcatccaa atacgatctt ggttatgcag atgtgccaca gaacatgcgg gcccatacat	1140
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<210> 9
<211> 1026
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> CDS
<222> (1)...(1026)

<400> 9

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1 5 10 15	
aac ccg ttc gcg gcc ccg cgc agg ccc tgg ttc ccc aga acc gac cct Asn Pro Phe Ala Ala Pro Arg Arg Pro Trp Phe Pro Arg Thr Asp Pro	96
20 25 30	
ttt ctg gcg atg cag gtg cag gaa tta acc cgc tcg atg gct aac ctg Phe Leu Ala Met Gln Val Gln Glu Leu Thr Arg Ser Met Ala Asn Leu	144
35 40 45	
acg ttc aag caa cgc cgg gac gcg cca cct gag ggg cca tcc gct aag Thr Phe Lys Gln Arg Arg Asp Ala Pro Pro Glu Gly Pro Ser Ala Lys	192
50 55 60	
aaa ccg aag aag gag gcc tcg caa aaa cag aaa ggg gga ggc caa ggg Lys Pro Lys Lys Glu Ala Ser Gln Lys Gln Lys Gly Gly Gly Gln Gly	240
65 70 75 80	
aag aag aag aac caa ggg aag aag gct aag aca ggg ccc cct Lys Lys Lys Asn Gln Gly Lys Lys Ala Lys Thr Gly Pro Pro	288
85 90 95	
aat ccg aag gca cag aat gga aac aag aag acc aac aag aaa cca Asn Pro Lys Ala Gln Asn Gly Asn Lys Lys Lys Thr Asn Lys Lys Pro	336
100 105 110	
ggc aag aga cag cgc atg gtc atg aaa ttg gaa tct gac aag acg ttc Gly Lys Arg Gln Arg Met Val Met Lys Leu Glu Ser Asp Lys Thr Phe	384
115 120 125	
cca atc atg ttg gaa ggg aag ata aac ggc tac gct tgt gtg gtc gga Pro Ile Met Leu Glu Gly Lys Ile Asn Gly Tyr Ala Cys Val Val Gly	432
130 135 140	
ggg aag tta ttc agg ccg atg cat gtg gaa ggc aag atc gac aac gac Gly Lys Leu Phe Arg Pro Met His Val Glu Gly Lys Ile Asp Asn Asp	480
145 150 155 160	
gtt ctg gcc gcg ctt aag acg aag aaa gca tcc aaa tac gat ctt gag Val Leu Ala Ala Leu Lys Thr Lys Lys Ala Ser Lys Tyr Asp Leu Glu	528
165 170 175	
tat gca gat gtg cca cag aac atg cgg gcc gat aca ttc aaa tac acc Tyr Ala Asp Val Pro Gln Asn Met Arg Ala Asp Thr Phe Lys Tyr Thr	576
180 185 190	
cat gag aaa ccc caa ggc tat tac agc tgg cat cat gga gca gtc caa His Glu Lys Pro Gln Gly Tyr Tyr Ser Trp His His Gly Ala Val Gln	624
195 200 205	
195 200 205	
tat gaa aat ggg cgt ttc acg gtg ccg aaa gga gtt ggg gcc aag gga Tyr Glu Asn Gly Arg Phe Thr Val Pro Lys Gly Val Gly Ala Lys Gly	672
210 215 220	

gac agc gga cga ccc att ctg gat aac cag gga cgg gtg gtc gct att			720
Asp Ser Gly Arg Pro Ile Leu Asp Asn Gln Gly Arg Val Val Ala Ile			
225	230	235	240
gtg ctg gga ggt gtg aat gaa gga tct agg aca gcc ctt tca gtc gtc			768
Val Leu Gly Gly Val Asn Glu Gly Ser Arg Thr Ala Leu Ser Val Val			
245	250	255	
atg tgg aac gag aag gga gtt acc gtg aag tat act ccg gag aac tgc			816
Met Trp Asn Glu Lys Gly Val Thr Val Lys Tyr Thr Pro Glu Asn Cys			
260	265	270	
gag caa tgg tca cta gtg acc acc atg tgt ctg ctc gcc aat gtg acg			864
Glu Gln Trp Ser Leu Val Thr Thr Met Cys Leu Leu Ala Asn Val Thr			
275	280	285	
ttc cca tgt gct caa cca cca att tgc tac gac aga aaa cca gca gag			912
Phe Pro Cys Ala Gln Pro Pro Ile Cys Tyr Asp Arg Lys Pro Ala Glu			
290	295	300	
act ttg gcc atg ctc agc gtt aac atc cct gct ggg agg atc agc cgt			960
Thr Leu Ala Met Leu Ser Val Asn Ile Pro Ala Gly Arg Ile Ser Arg			
305	310	315	320
aat tat tat aat tgg ctt ggt gct ggc tac tat tgt ggc cat gta cgt			1008
Asn Tyr Tyr Asn Trp Leu Gly Ala Gly Tyr Tyr Cys Gly His Val Arg			
325	330	335	
gct gac caa cca gaa aca			1026
Ala Asp Gln Pro Glu Thr			
340			

<210> 10
<211> 342
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

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<400> 10
Met Phe Pro Phe Gln Pro Met Tyr Pro Met Gln Pro Met Pro Tyr Arg
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Asn Pro Phe Ala Ala Pro Arg Arg Pro Trp Phe Pro Arg Thr Asp Pro
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Phe Leu Ala Met Gln Val Gln Glu Leu Thr Arg Ser Met Ala Asn Leu
   35         40           45
Thr Phe Lys Gln Arg Arg Asp Ala Pro Pro Glu Gly Pro Ser Ala Lys
   50         55           60
Lys Pro Lys Lys Glu Ala Ser Gln Lys Gln Lys Gly Gly Gln Gln Gly
   65         70           75           80

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Lys Lys Lys Lys Asn Gln Gly Lys Lys Ala Lys Thr Gly Pro Pro
 85 90 95
 Asn Pro Lys Ala Gln Asn Gly Asn Lys Lys Lys Thr Asn Lys Lys Pro
 100 105 110
 Gly Lys Arg Gln Arg Met Val Met Lys Leu Glu Ser Asp Lys Thr Phe
 115 120 125
 Pro Ile Met Leu Glu Gly Lys Ile Asn Gly Tyr Ala Cys Val Val Gly
 130 135 140
 Gly Lys Leu Phe Arg Pro Met His Val Glu Gly Lys Ile Asp Asn Asp
 145 150 155 160
 Val Leu Ala Ala Leu Lys Thr Lys Lys Ala Ser Lys Tyr Asp Leu Glu
 165 170 175
 Tyr Ala Asp Val Pro Gln Asn Met Arg Ala Asp Thr Phe Lys Tyr Thr
 180 185 190
 His Glu Lys Pro Gln Gly Tyr Ser Trp His His Gly Ala Val Gln
 195 200 205
 Tyr Glu Asn Gly Arg Phe Thr Val Pro Lys Gly Val Gly Ala Lys Gly
 210 215 220
 Asp Ser Gly Arg Pro Ile Leu Asp Asn Gln Gly Arg Val Val Ala Ile
 225 230 235 240
 Val Leu Gly Gly Val Asn Glu Gly Ser Arg Thr Ala Leu Ser Val Val
 245 250 255
 Met Trp Asn Glu Lys Gly Val Thr Val Lys Tyr Thr Pro Glu Asn Cys
 260 265 270
 Glu Gln Trp Ser Leu Val Thr Thr Met Cys Leu Leu Ala Asn Val Thr
 275 280 285
 Phe Pro Cys Ala Gln Pro Pro Ile Cys Tyr Asp Arg Lys Pro Ala Glu
 290 295 300
 Thr Leu Ala Met Leu Ser Val Asn Ile Pro Ala Gly Arg Ile Ser Arg
 305 310 315 320
 Asn Tyr Tyr Asn Trp Leu Gly Ala Gly Tyr Tyr Cys Gly His Val Arg
 325 330 335
 Ala Asp Gln Pro Glu Thr
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<210> 11
 <211> 6989
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence; Note =
 synthetic construct

<400> 11

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aggtagaagc	caagcaggtc	actgataatg	accatgctaa	tgccagagcg	tttcgcate	180
tggcttcaa	actgatcgaa	acggaggtgg	acccatccga	cacgatccctt	gacattggaa	240
gtgcggccgc	ccgcagaatg	tattctaagc	acaagtatca	ttgtatctgt	ccgatgagat	.300
gtgcggaga	tccggacaga	ttgtataagt	atgcaactaa	gctgaagaaa	aactgttaagg	360
aaataactga	taaggaattg	gacaagaaaa	tgaaggagct	cgccgcccgc	atgagcgacc	420
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aagtgcgtgt	ttaccaggat	gtatacgcgg	ttgacggacc	ctataactct	ctacggctaa	540
cctgaatgga	ctacgacata	gtctagtccg	ccaagatgtc	actagtgacc	accatgtgtc	600

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at	ggg	ttt	aa	gg	gg	1860
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ttt	cc	ttt	cc	tt	gt	1980
ttt	cc	ttt	cc	tt	tc	2040
cattt	gt	ct	gt	cc	tc	2100
ccttggatca	cct	atgg	aa	at	tt	2160
tggccgc	ttt	gt	atgg	cc	gt	2220
tagt	cat	ggc	cc	cc	gg	2280
aagcgg	gg	at	cc	cc	cc	2340
gcata	ac	aa	aa	at	gt	2400
actaca	aa	aa	aa	at	gt	2460
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aat	ct	gt	cc	tt	tt	2640
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gctc	at	ttt	ttt	ttt	ttt	2940
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ctt	ttt	ttt	ttt	ttt	ttt	3660
aaaa	aaaa	aaaa	aaaa	aaaa	aaaa	3720
ggcgtt	cc	cc	cc	cc	cc	3780
catcg	cc	cc	cc	cc	cc	3840
acag	cc	cc	cc	cc	cc	3900

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tagcgggtgt	tttttgttt	gcaagcagca	gattacgcgc	agaaaaaaAG	gatctcaaga	5880
agatccTTG	atctttcta	cggggtctga	cgctcagtgg	aacgaaaact	cacgttaagg	5940
gattttggc	atgaacaata	aaactgtctg	cttacataaa	cagtaataca	agggggtgtt	6000
tgagccat	tcaacgggaa	acgtcttgc	cgaggccgc	attaaattcc	aacatggatg	6060
ctgatttata	tgggtataaa	tgggtcgc	ataatgtc	gcaatcagg	gcgacaatct	6120
atcgattgt	tgggaagccc	gatgcgc	agttgtttct	gaaacatggc	aaaggttagcg	6180
ttgccaatga	tgttacagat	gagatggta	gactaaactg	gctgacggaa	tttatgcctc	6240
ttccgaccat	caagcattt	atccgtactc	ctgtatgtgc	atggttactc	accactgcga	6300
tcccccggaa	aacagcattc	caggattag	aagaatatcc	tgattcagg	gaaaatattg	6360
ttgatgcgt	ggcagtgtt	ctgcgcgg	tgcatcgat	tcctgttgt	aattgtcctt	6420
ttaacagcga	tcgcgtattt	ctgtctcg	aggcgcaatc	acgaatgaat	aacggtttgg	6480
ttgatgcgt	tgatTTGAT	gacgacgt	atggctggcc	tgttgaacaa	gtctggaaAG	6540
aaatgcataa	gttttgc	ttctcacccg	attcagtcgt	cactcatgg	gatttctcac	6600
ttgataacct	tatTTTGAC	gagggaaat	taatagggtt	tattgtatgtt	ggacgagtcg	6660
gaatcgcaga	ccgataccag	gatctgcca	tcctatggaa	ctgcctcggt	gagttttctc	6720
cttcattaca	gaaacggctt	tttcaaaat	atggtattga	taatcctgtat	atgaataaaat	6780
tgcagtTTCA	tttgatgtc	gatgatTTT	tctaagaatt	ctcatgttt	acagcttac	6840
atcgataagc	ttaatgcgg	tagttatca	cagttaaatt	gctaaccgc	tcaggcaccg	6900
tgtatgaaat	ctaacaatgc	gctcatcg	atccctggca	ccgtcaccct	ggatgtgtc	6960
tagaggatcc	ctaatacgc	tcactatag				6989

<210> 12
<211> 2943
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> CDS
<222> (1) . . . (2943)

<400> 12
 atg tca cta gtg acc acc atg tgt ctg ctc gcc aat gtg acg ttc cca 48
 Met Ser Leu Val Thr Thr Met Cys Leu Leu Ala Asn Val Thr Phe Pro
 1 5. 10 15

 tgt gct caa cca cca att tgc tac gac aga aaa cca gca gag act ttg 96
 Cys Ala Gln Pro Pro Ile Cys Tyr Asp Arg Lys Pro Ala Glu Thr Leu
 20 25 30

 gcc atg ctc agc gtt aac gtt gac aac ccg ggc tac gat gag ctg ctg 144
 Ala Met Leu Ser Val Asn Val Asp Asn Pro Gly Tyr Asp Glu Leu Leu
 35 40 45

 gaa gca gct gtt aag tgc ccc gga agg aaa agg aga tcc acc gag gag 192
 Glu Ala Ala Val Lys Cys Pro Gly Arg Lys Arg Arg Ser Thr Glu Glu
 50 55 60

 ctg ttt aag gag tat aag cta acg cgc cct tac atg gcc aga tgc atc 240
 Leu Phe Lys Glu Tyr Lys Leu Thr Arg Pro Tyr Met Ala Arg Cys Ile
 65 70 75 80

 aga tgt gca gtt ggg agc tgc cat agt cca ata gca atc gag gca gta 288
 Arg Cys Ala Val Gly Ser Cys His Ser Pro Ile Ala Ile Glu Ala Val
 85 90 95

 aag agc gac ggg cac gac ggt tat gtt aga ctt cag act tcc tcg cag 336
 Lys Ser Asp Gly His Asp Gly Tyr Val Arg Leu Gln Thr Ser Ser Gln
 100 105 110

 tat ggc ctg gat tcc tcc ggc aac tta aag ggc agg acc atg cgg tat 384
 Tyr Gly Leu Asp Ser Ser Gly Asn Leu Lys Gly Arg Thr Met Arg Tyr
 115 120 125

 gac atg cac ggg acc att aaa gag ata cca cta cat caa gtg tca ctc 432
 Asp Met His Gly Thr Ile Lys Glu Ile Pro Leu His Gln Val Ser Leu
 130 135 140

 cat aca tct cgc ccg tgt cac att gtg gat ggg cac ggt tat ttc ctg 480
 His Thr Ser Arg Pro Cys His Ile Val Asp Gly His Gly Tyr Phe Leu
 145 150 155 160
 ctt gcc agg tgc ccg gca ggg gac tcc atc acc atg gaa ttt aag aaa 528
 Leu Ala Arg Cys Pro Ala Gly Asp Ser Ile Thr Met Glu Phe Lys Lys
 165 170 175

gat tcc gtc aca cac tcc tgc tcg gtg ccg tat gaa gtg aaa ttt aat Asp Ser Val Thr His Ser Cys Ser Val Pro Tyr Glu Val Lys Phe Asn 180 185 190	576
cct gta ggc aga gaa ctc tat act cat ccc cca gaa cac gga gta gag Pro Val Gly Arg Glu Leu Tyr Thr His Pro Pro Glu His Gly Val Glu 195 200 205	624
caa gcg tgc caa gtc tac gca cat gat gca cag aac aga gga gct tat Gln Ala Cys Gln Val Tyr Ala His Asp Ala Gln Asn Arg Gly Ala Tyr 210 215 220	672
gtc gag atg cac ctc cca ggc tca gaa gtg gac agc agt ttg gtt tcc Val Glu Met His Leu Pro Gly Ser Glu Val Asp Ser Ser Leu Val Ser 225 230 235 240	720
ttg agc ggc agt tca gtc acc gtg aca cct cct gtt ggg act agc gcc Leu Ser Gly Ser Ser Val Thr Val Thr Pro Pro Val Gly Thr Ser Ala 245 250 255	768
ctg gtg gaa tgc gag tgt ggc ggc aca aag atc tcc aag acc atc aac Leu Val Glu Cys Glu Cys Gly Gly Thr Lys Ile Ser Lys Thr Ile Asn 260 265 270	816
aag aca aaa cag ttc agc cag tgc aca aag aag gag cag tgc aga gca Lys Thr Lys Gln Phe Ser Gln Cys Thr Lys Lys Glu Gln Cys Arg Ala 275 280 285	864
tat cgg ctg cag aac gat aag tgg gtg tat aat tct gac aaa ctg ccc Tyr Arg Leu Gln Asn Asp Lys Trp Val Tyr Asn Ser Asp Lys Leu Pro 290 295 300	912
aaa gca gcg gga gcc acc tta aaa gga aaa ctg cat gtc cca ttc ttg Lys Ala Ala Gly Ala Thr Leu Lys Gly Lys Leu His Val Pro Phe Leu 305 310 315 320	960
ctg gca gac ggc aaa tgc acc gtg cct cta gca cca gaa cct atg ata Leu Ala Asp Gly Lys Cys Thr Val Pro Leu Ala Pro Glu Pro Met Ile 325 330 335	1008
acc ttc ggt ttc aga tca gtg tca ctg aaa ctg cac cct aag aat ccc Thr Phe Gly Phe Arg Ser Val Ser Leu Lys Leu His Pro Lys Asn Pro 340 345 350	1056
aca tat cta acc acc cgc caa ctt gct gat gag cct cac tac acg cat Thr Tyr Leu Thr Thr Arg Gln Leu Ala Asp Glu Pro His Tyr Thr His 355 360 365	1104
gag ctc ata tct gaa cca gct gtt agg aat ttt acc gtc acc gga aaa Glu Leu Ile Ser Glu Pro Ala Val Arg Asn Phe Thr Val Thr Gly Lys 370 375 380	1152
ggg tgg gag ttt gta tgg gga aac cac ccg ccg aaa agg ttt tgg gca Gly Trp Glu Phe Val Trp Gly Asn His Pro Pro Lys Arg Phe Trp Ala 385 390 395 400	1200

cag gaa aca gca ccc gga aat cca cat ggg cta ccg cac gag gtg ata Gln Glu Thr Ala Pro Gly Asn Pro His Gly Leu Pro His Glu Val Ile 405 410 415	1248
act cat tat tac cac aga tac cct atg tcc acc atc ctg ggt ttg tca Thr His Tyr Tyr His Arg Tyr Pro Met Ser Thr Ile Leu Gly Leu Ser 420 425 430	1296
att tgt gcc gcc att gca acc gtt tcc gtt gca gcg tct acc tgg ctg Ile Cys Ala Ala Ile Ala Thr Val Ser Val Ala Ala Ser Thr Trp Leu 435 440 445	1344
ttt tgc aga tct aga gtt gcg tgc cta act cct tac cgg cta aca cct Phe Cys Arg Ser Arg Val Ala Cys Leu Thr Pro Tyr Arg Leu Thr Pro 450 455 460	1392
aac gct agg ata cca ttt tgt ctg gct gtg ctt tgc tgc gcc cgc act Asn Ala Arg Ile Pro Phe Cys Leu Ala Val Leu Cys Cys Ala Arg Thr 465 470 475 480	1440
gcc cgg gcc gag acc acc tgg gag tcc ttg gat cac cta tgg aac aat Ala Arg Ala Glu Thr Thr Trp Glu Ser Leu Asp His Leu Trp Asn Asn 485 490 495	1488
aac caa cag atg ttc tgg att caa ttg ctg atc cct ctg gcc gcc ttg Asn Gln Gln Met Phe Trp Ile Gln Leu Leu Ile Pro Leu Ala Ala Leu 500 505 510	1536
atc gta gtg act cgc ctg ctc agg tgc gtg tgc tgt gtc gtg cct ttt Ile Val Val Thr Arg Leu Leu Arg Cys Val Cys Cys Val Val Pro Phe 515 520 525	1584
tta gtc atg gcc ggc gca ggc gcc ggc tac gag cac gcg acc Leu Val Met Ala Gly Ala Ala Gly Ala Tyr Glu His Ala Thr 530 535 540	1632
acg atg ccg agc caa gcg gga atc tcg tat aac act ata gtc aac aga Thr Met Pro Ser Gln Ala Gly Ile Ser Tyr Asn Thr Ile Val Asn Arg 545 550 555 560	1680
gca ggc tac gca cca ctc cct atc agc ata aca cca aca aag atc aag Ala Gly Tyr Ala Pro Leu Pro Ile Ser Ile Thr Pro Thr Lys Ile Lys 565 570 575	1728
ctg ata cct aca gtg aac ttg gag tac gtc acc tgc cac tac aaa aca Leu Ile Pro Thr Val Asn Leu Glu Tyr Val Thr Cys His Tyr Lys Thr 580 585 590	1776
gga atg gat tca cca gcc atc aaa tgc tgc gga tct cag gaa tgc act Gly Met Asp Ser Pro Ala Ile Lys Cys Cys Gly Ser Gln Glu Cys Thr 595 600 605	1824
cca act tac agg cct gat gaa cag tgc aaa gtc ttc aca ggg gtt tac Pro Thr Tyr Arg Pro Asp Glu Gln Cys Lys Val Phe Thr Gly Val Tyr 610 615 620	1872

ccg ttc atg tgg ggt gca tat tgc ttt tgc gac act gag aac acc Pro Phe Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Thr Glu Asn Thr 625 630 635 640	1920
caa gtc agc aag gcc tac gta atg aaa tct gac gac tgc ctt gcg gat Gln Val Ser Lys Ala Tyr Val Met Lys Ser Asp Asp Cys Leu Ala Asp 645 650 655	1968
cat gct gaa gca tat aaa gcg cac aca gcc tca gtg cag gcg ttc ctc His Ala Glu Ala Tyr Lys Ala His Thr Ala Ser Val Gln Ala Phe Leu 660 665 670	2016
aac atc aca gtg gga gaa cac tct att gtg act acc gtg tat gtg aat Asn Ile Thr Val Gly Glu His Ser Ile Val Thr Thr Val Tyr Val Asn 675 680 685	2064
gga gaa act cct gtg aat ttc aat ggg gtc aaa tta act gca ggt ccg Gly Glu Thr Pro Val Asn Phe Asn Gly Val Lys Leu Thr Ala Gly Pro 690 695 700	2112
ctt tcc aca gct tgg aca ccc ttt gat cgc aaa atc gtg cag tat gcc Leu Ser Thr Ala Trp Thr Pro Phe Asp Arg Lys Ile Val Gln Tyr Ala 705 710 715 720	2160
ggg gag atc tat aat tat gat ttt cct gag tat ggg gca gga caa cca Gly Glu Ile Tyr Asn Tyr Asp Phe Pro Glu Tyr Gly Ala Gly Gln Pro 725 730 735	2208
gga gca ttt gga gat ata caa tcc aga aca gtc tca agc tca gat ctg Gly Ala Phe Gly Asp Ile Gln Ser Arg Thr Val Ser Ser Asp Leu 740 745 750	2256
tat gcc aat acc aac cta gtg ctg cag aga ccc aaa gca gga gcg atc Tyr Ala Asn Thr Asn Leu Val Leu Gln Arg Pro Lys Ala Gly Ala Ile 755 760 765	2304
cac gtg cca tac act cag gca cct tcg ggt ttt gag caa tgg aag aaa His Val Pro Tyr Thr Gln Ala Pro Ser Gly Phe Glu Gln Trp Lys Lys 770 775 780	2352
gat aaa gct cca tca ttg aaa ttt acc gcc cct ttc gga tgc gaa ata Asp Lys Ala Pro Ser Leu Lys Phe Thr Ala Pro Phe Gly Cys Glu Ile 785 790 795 800	2400
tat aca aac ccc att cgc gcc gaa aac tgt act gta ggg tca att cca Tyr Thr Asn Pro Ile Arg Ala Glu Asn Cys Thr Val Gly Ser Ile Pro 805 810 815	2448
tta gcc ttt gac att ccc gac gcc ttg ttc acc agg gtg tca gaa aca Leu Ala Phe Asp Ile Pro Asp Ala Leu Phe Thr Arg Val Ser Glu Thr 820 825 830	2496
ccg aca ctt tca gcg gcc gaa tgc act ctt aac gag tgc gtg tat tct Pro Thr Leu Ser Ala Ala Glu Cys Thr Leu Asn Glu Cys Val Tyr Ser 835 840 845	2544

<210> 13
<211> 981
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 13
 Met Ser Leu Val Thr Thr Met Cys Leu Leu Ala Asn Val Thr Phe Pro
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 Cys Ala Gln Pro Pro Ile Cys Tyr Asp Arg Lys Pro Ala Glu Thr Leu
 20 25 30
 Ala Met Leu Ser Val Asn Val Asp Asn Pro Gly Tyr Asp Glu Leu Leu
 35 40 45

Glu Ala Ala Val Lys Cys Pro Gly Arg Arg Ser Thr Glu Glu
 50 55 60
 Leu Phe Lys Glu Tyr Lys Leu Thr Arg Pro Tyr Met Ala Arg Cys Ile
 65 70 75 80
 Arg Cys Ala Val Gly Ser Cys His Ser Pro Ile Ala Ile Glu Ala Val
 85 90 95
 Lys Ser Asp Gly His Asp Gly Tyr Val Arg Leu Gln Thr Ser Ser Gln
 100 105 110
 Tyr Gly Leu Asp Ser Ser Gly Asn Leu Lys Gly Arg Thr Met Arg Tyr
 115 120 125
 Asp Met His Gly Thr Ile Lys Glu Ile Pro Leu His Gln Val Ser Leu
 130 135 140
 His Thr Ser Arg Pro Cys His Ile Val Asp Gly His Gly Tyr Phe Leu
 145 150 155 160
 Leu Ala Arg Cys Pro Ala Gly Asp Ser Ile Thr Met Glu Phe Lys Lys
 165 170 175
 Asp Ser Val Thr His Ser Cys Ser Val Pro Tyr Glu Val Lys Phe Asn
 180 185 190
 Pro Val Gly Arg Glu Leu Tyr Thr His Pro Pro Glu His Gly Val Glu
 195 200 205
 Gln Ala Cys Gln Val Tyr Ala His Asp Ala Gln Asn Arg Gly Ala Tyr
 210 215 220
 Val Glu Met His Leu Pro Gly Ser Glu Val Asp Ser Ser Leu Val Ser
 225 230 235 240
 Leu Ser Gly Ser Ser Val Thr Val Thr Pro Pro Val Gly Thr Ser Ala
 245 250 255
 Leu Val Glu Cys Glu Cys Gly Gly Thr Lys Ile Ser Lys Thr Ile Asn
 260 265 270
 Lys Thr Lys Gln Phe Ser Gln Cys Thr Lys Lys Glu Gln Cys Arg Ala
 275 280 285
 Tyr Arg Leu Gln Asn Asp Lys Trp Val Tyr Asn Ser Asp Lys Leu Pro
 290 295 300
 Lys Ala Ala Gly Ala Thr Leu Lys Gly Lys Leu His Val Pro Phe Leu
 305 310 315 320
 Leu Ala Asp Gly Lys Cys Thr Val Pro Leu Ala Pro Glu Pro Met Ile
 325 330 335
 Thr Phe Gly Phe Arg Ser Val Ser Leu Lys Leu His Pro Lys Asn Pro
 340 345 350
 Thr Tyr Leu Thr Thr Arg Gln Leu Ala Asp Glu Pro His Tyr Thr His
 355 360 365
 Glu Leu Ile Ser Glu Pro Ala Val Arg Asn Phe Thr Val Thr Gly Lys
 370 375 380
 Gly Trp Glu Phe Val Trp Gly Asn His Pro Pro Lys Arg Phe Trp Ala
 385 390 395 400
 Gln Glu Thr Ala Pro Gly Asn Pro His Gly Leu Pro His Glu Val Ile
 405 410 415
 Thr His Tyr Tyr His Arg Tyr Pro Met Ser Thr Ile Leu Gly Leu Ser
 420 425 430
 Ile Cys Ala Ala Ile Ala Thr Val Ser Val Ala Ala Ser Thr Trp Leu
 435 440 445
 Phe Cys Arg Ser Arg Val Ala Cys Leu Thr Pro Tyr Arg Leu Thr Pro
 450 455 460
 Asn Ala Arg Ile Pro Phe Cys Leu Ala Val Leu Cys Cys Ala Arg Thr
 465 470 475 480

Ala Arg Ala Glu Thr Thr Trp Glu Ser Leu Asp His Leu Trp Asn Asn
 485 490 495
 Asn Gln Gln Met Phe Trp Ile Gln Leu Leu Ile Pro Leu Ala Ala Leu
 500 505 510
 Ile Val Val Thr Arg Leu Leu Arg Cys Val Cys Cys Val Val Pro Phe
 515 520 525
 Leu Val Met Ala Gly Ala Ala Gly Ala Tyr Glu His Ala Thr
 530 535 540
 Thr Met Pro Ser Gln Ala Gly Ile Ser Tyr Asn Thr Ile Val Asn Arg
 545 550 555 560
 Ala Gly Tyr Ala Pro Leu Pro Ile Ser Ile Thr Pro Thr Lys Ile Lys
 565 570 575
 Leu Ile Pro Thr Val Asn Leu Glu Tyr Val Thr Cys His Tyr Lys Thr
 580 585 590
 Gly Met Asp Ser Pro Ala Ile Lys Cys Cys Gly Ser Gln Glu Cys Thr
 595 600 605
 Pro Thr Tyr Arg Pro Asp Glu Gln Cys Lys Val Phe Thr Gly Val Tyr
 610 615 620
 Pro Phe Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Thr Glu Asn Thr
 625 630 635 640
 Gln Val Ser Lys Ala Tyr Val Met Lys Ser Asp Asp Cys Leu Ala Asp
 645 650 655
 His Ala Glu Ala Tyr Lys Ala His Thr Ala Ser Val Gln Ala Phe Leu
 660 665 670
 Asn Ile Thr Val Gly Glu His Ser Ile Val Thr Thr Val Tyr Val Asn
 675 680 685
 Gly Glu Thr Pro Val Asn Phe Asn Gly Val Lys Leu Thr Ala Gly Pro
 690 695 700
 Leu Ser Thr Ala Trp Thr Pro Phe Asp Arg Lys Ile Val Gln Tyr Ala
 705 710 715 720
 Gly Glu Ile Tyr Asn Tyr Asp Phe Pro Glu Tyr Gly Ala Gly Gln Pro
 725 730 735
 Gly Ala Phe Gly Asp Ile Gln Ser Arg Thr Val Ser Ser Asp Leu
 740 745 750
 Tyr Ala Asn Thr Asn Leu Val Leu Gln Arg Pro Lys Ala Gly Ala Ile
 755 760 765
 His Val Pro Tyr Thr Gln Ala Pro Ser Gly Phe Glu Gln Trp Lys Lys
 770 775 780
 Asp Lys Ala Pro Ser Leu Lys Phe Thr Ala Pro Phe Gly Cys Glu Ile
 785 790 795 800
 Tyr Thr Asn Pro Ile Arg Ala Glu Asn Cys Thr Val Gly Ser Ile Pro
 805 810 815
 Leu Ala Phe Asp Ile Pro Asp Ala Leu Phe Thr Arg Val Ser Glu Thr
 820 825 830
 Pro Thr Leu Ser Ala Ala Glu Cys Thr Leu Asn Glu Cys Val Tyr Ser
 835 840 845
 Ser Asp Phe Gly Gly Ile Ala Thr Val Lys Tyr Ser Ala Ser Lys Ser
 850 855 860
 Gly Lys Cys Ala Val His Val Pro Ser Gly Thr Ala Thr Leu Lys Glu
 865 870 875 880
 Ala Ala Val Glu Leu Thr Glu Gln Gly Ser Ala Thr Ile His Phe Ser
 885 890 895
 Thr Ala Asn Ile His Pro Glu Phe Arg Leu Gln Ile Cys Thr Ser Tyr
 900 905 910

Val Thr Cys Lys Gly Asp Cys His Pro Pro Lys Asp His Ile Val Thr
 915 920 925
 His Pro Gln Tyr His Ala Gln Thr Phe Thr Ala Ala Val Ser Lys Thr
 930 935 940
 Ala Trp Thr Trp Leu Thr Ser Leu Leu Gly Gly Ser Ala Val Ile Ile
 945 950 955 960
 Ile Ile Gly Leu Val Leu Ala Thr Ile Val Ala Met Tyr Val Leu Thr
 965 970 975
 Asn Gln Lys His Asn
 980

<210> 14
<211> 12379
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 14

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aggtagaagc	caagcaggtc	actgataatg	accatgtcaa	tgccagagcg	tttcgcac	180
tggcttcaaa	actgatcgaa	acggagggtgg	acccatccga	cacgatcctt	gacattggaa	240
gtggccccgc	ccgcagaatg	tattctaagc	acaagtatca	ttgtatctgt	ccgatgagat	300
gtgcggaaaga	tccggacaga	ttgtataagt	atgcaactaa	gctgaagaaa	aactgtaaagg	360
aaataactga	taaggaattt	gacaagaaaa	tgaaggagct	cgccgcgtc	atgagcgacc	420
ctgaccttgg	aactgagact	atgtcctcc	acgacgacga	gtcgtgtcgc	tacaaagggc	480
aatgcgtgt	ttaccaggat	gtatacgcgg	ttgacggacc	gacaagtctc	tatcaccaag	540
ccaataaggg	agtttagagtc	gcctacttgg	taggctttga	caccaccctt	tttatgttta	600
agaacttggc	tggagcatat	ccatcatact	ctaccaactg	ggccgcacgaa	accgtgttta	660
cggctcgtaa	cataggccta	tgcagctctg	acgttatgg	gcggtcacgt	agagggatgt	720
ccatttcttag	aaagaagat	ttgaaaccat	ccaacaatgt	tctattctct	gttggctcga	780
ccatctacca	cgagaagagg	gacttactga	ggagctggca	cctgcccgtct	gtatccact	840
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cgtgcacccg	cgagggat	ttgtgtcgtca	aagtgcacaga	cacattcaac	ggggagaggg	1020
tctctttcc	cgtgtgcacg	tatgtgccag	ctacatttgt	tgaccaaatg	actggcatac	1080
tggcaacaga	tgtcagtgcg	gacgacgcgc	aaaaactgtct	ggttgggctc	aaccagcgta	1140
tagtgcgtca	cggtcgcacc	cagagaaaca	ccaataccat	gaaaaattac	cttttgc	1200
tagtggccca	ggcatttgct	aggtgggcaa	aggaatataa	ggaagatcaa	gaagatgaaa	1260
ggccactagg	actacgagat	agacagttag	tcatggggt	ttgttgggct	tttagaaggc	1320
acaagataac	atctatttat	aagcgc	ccgg	atacccaa	gtaacagcg	1380
attccactc	attcgtgtc	cccaggatag	gcagtaacac	attggagatc	gggctgagaa	1440
caagaatcag	aaaaatgtta	gaggagcaca	aggagccgtc	acctctcatt	accgcgagg	1500
acgtacaaga	agctaagtgc	gcagccgtat	agcgtaagga	ggtgcgtgaa	gccgaggagt	1560
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tgccaggg	acatgcaata	cccgtccagg	acttcaagc	tctgagtggaa	agtgcacca	1920
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gagcgtgaa	cactgatgaa	aatattaca	aaactgtcaa	gcccagcgag	cacgacggcg	2040

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<210> 15
<211> 1323
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

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<222> (1)...(1323)

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Met Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly							
1	5	10	15				
atg gat ggc cca aag gtt aaa caa tgg ccg tta aca gaa gtg aaa ata							96
Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Val Lys Ile							
20	25	30					
aaa gca tta aca gca att tgt gaa gaa atg gaa aag gaa gga aaa att							144
Lys Ala Leu Thr Ala Ile Cys Glu Glu Met Glu Lys Glu Gly Lys Ile							
35	40	45					
aca aaa att ggg cct gaa aat cca tat aac act cca ata ttc gcc ata							192
Thr Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Ile Phe Ala Ile							
50	55	60					
aaa aag gaa gac agc act aag tgg aga aaa tta gta gat ttc agg gaa							240
Lys Lys Glu Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu							
65	70	75	80				
ctc aat aaa aga act caa gac ttt tgg gag gtt caa tta gga ata cca							288
Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro							
85	90	95					
cac cca gca ggg tta aaa aag aaa aaa tca gtg aca gta ctg gat gtg							336
His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val							
100	105	110					
gga gat gca tat ttt tca gtt cct tta gat gaa ggc ttc agg aaa tat							384
Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Gly Phe Arg Lys Tyr							
115	120	125					

act gca ttc acc ata cct agt ata aac aat gaa aca cca ggg att aga		432
Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg		
130	135	140
tat caa tat aat gtg ctt cca caa gga tgg aaa ggg tca cca gca ata		480
Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile		
145	150	155
ttc cag gct agc atg aca aaa atc cta gag ccc ttt aga gct aaa aat		528
Phe Gln Ala Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Ala Lys Asn		
165	170	175
cca gaa ata gtc atc tat caa cat atg gcg gca ttg tat gta gga tct		576
Pro Glu Ile Val Ile Tyr Gln His Met Ala Ala Leu Tyr Val Gly Ser		
180	185	190
gac tta gaa ata ggg caa cat aga gca aaa ata gaa gag tta aga gaa		624
Asp Leu Glu Ile Gly Gln His Arg Ala Lys Ile Glu Glu Leu Arg Glu		
195	200	205
cat cta tta aag tgg gga ttt acc aca cca gac aaa aaa cat cag aaa		672
His Leu Leu Lys Trp Gly Phe Thr Thr Pro Asp Lys Lys His Gln Lys		
210	215	220
gaa ccc cca ttt ctt tgg atg ggg tat gaa ctc cat cct gac aaa tgg		720
Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp		
225	230	235
aca gta cag cct ata cag ctg cca gaa aaa gat agc tgg act gtc aat		768
Thr Val Gln Pro Ile Gln Leu Pro Glu Lys Asp Ser Trp Thr Val Asn		
245	250	255
gac ata cag aag tta gtg gga aaa tta aac tgg aca agt cag att tac		816
Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Thr Ser Gln Ile Tyr		
260	265	270
cca ggg att aaa gta agg caa ctt tgt aag ctc ctt agg ggg acc aaa		864
Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys		
275	280	285
gca cta aca gac ata gta cca cta act gaa gaa gca gaa tta gaa ttg		912
Ala Leu Thr Asp Ile Val Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu		
290	295	300
gca gag aac agg gaa att cta aaa gaa cca gtg cat gga gta tat tat		960
Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr		
305	310	315
gac cca tca aaa gac ttg ata gct gaa ata cag aaa cag ggg gat gac		1008
Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Asp Asp		
325	330	335
caa tgg aca tat caa att tac caa gaa cca ttc aaa aac ctg aag aca		1056
Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr		
340	345	350

gga aag tat gca aaa agg agg act acc cac act aat gat gta aaa cag	1104
Gly Lys Tyr Ala Lys Arg Arg Thr Thr His Thr Asn Asp Val Lys Gln	
355	360
	365
tta aca gag gca gtg caa aaa ata tcc ttg gaa agc ata gta aca tgg	1152
Leu Thr Glu Ala Val Gln Lys Ile Ser Leu Glu Ser Ile Val Thr Trp	
370	375
	380
gga aag act cct aaa ttt aga cta ccc atc caa aaa gaa aca tgg gaa	1200
Gly Lys Thr Pro Lys Phe Arg Leu Pro Ile Gln Lys Glu Thr Trp Glu	
385	390
	395
	400
ata tgg tgg aca gac tat tgg caa gcc aca tgg att cct gag tgg gag	1248
Ile Trp Trp Thr Asp Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu	
405	410
	415
ttt gtt aat acc cct ccc cta gta aaa cta tgg tac cag cta gaa aaa	1296
Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys	
420	425
	430
gaa ccc ata gca gga gca gaa act ttc	1323
Glu Pro Ile Ala Gly Ala Glu Thr Phe	
435	440

<210> 16
 <211> 441
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence; Note =
 synthetic construct

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 Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Val Lys Ile
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 Lys Ala Leu Thr Ala Ile Cys Glu Glu Met Glu Lys Glu Gly Lys Ile
 35 40 45
 Thr Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Ile Phe Ala Ile
 50 55 60
 Lys Lys Glu Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu
 65 70 75 80
 Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro
 85 90 95
 His Pro Ala Gly Leu Lys Lys Ser Val Thr Val Leu Asp Val
 100 105 110
 Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Gly Phe Arg Lys Tyr
 115 120 125
 Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg
 130 135 140

Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile
 145 150 155 160
 Phe Gln Ala Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Ala Lys Asn
 165 170 175
 Pro Glu Ile Val Ile Tyr Gln His Met Ala Ala Leu Tyr Val Gly Ser
 180 185 190
 Asp Leu Glu Ile Gly Gln His Arg Ala Lys Ile Glu Glu Leu Arg Glu
 195 200 205
 His Leu Leu Lys Trp Gly Phe Thr Thr Pro Asp Lys Lys His Gln Lys
 210 215 220
 Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp
 225 230 235 240
 Thr Val Gln Pro Ile Gln Leu Pro Glu Lys Asp Ser Trp Thr Val Asn
 245 250 255
 Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Thr Ser Gln Ile Tyr
 260 265 270
 Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys
 275 280 285
 Ala Leu Thr Asp Ile Val Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu
 290 295 300
 Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr
 305 310 315 320
 Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Asp Asp
 325 330 335
 Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr
 340 345 350
 Gly Lys Tyr Ala Lys Arg Arg Thr Thr His Thr Asn Asp Val Lys Gln
 355 360 365
 Leu Thr Glu Ala Val Gln Lys Ile Ser Leu Glu Ser Ile Val Thr Trp
 370 375 380
 Gly Lys Thr Pro Lys Phe Arg Leu Pro Ile Gln Lys Glu Thr Trp Glu
 385 390 395 400
 Ile Trp Trp Thr Asp Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu
 405 410 415
 Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys
 420 425 430
 Glu Pro Ile Ala Gly Ala Glu Thr Phe
 435 440

<210> 17

<211> 13584

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 17

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 Gly Thr Leu Gly Phe Trp Met Ile Ile Ile Cys Arg Val Val Gly Asn
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ttg aac ttg tgg gtc aca gtc tat tat ggg gta cct gtg tgg aaa gaa 144
 Leu Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu
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<220>
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synthetic construct

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 35 40 45
 Ala Lys Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Lys
 50 55 60
 Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
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 Asn Pro Arg Glu Ile Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met
 85 90 95
 Trp Lys Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
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 Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
 115 120 125
 Thr Leu Asn Cys Thr Asn Ala Pro Ala Tyr Asn Asn Ser Met His Gly
 130 135 140
 Glu Met Lys Asn Cys Ser Phe Asn Thr Thr Thr Glu Ile Arg Asp Arg
 145 150 155 160
 Lys Gln Lys Ala Tyr Ala Leu Phe Tyr Lys Pro Asp Val Val Pro Leu
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 Asn Arg Arg Glu Glu Asn Asn Gly Thr Gly Glu Tyr Ile Leu Ile Asn
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 Cys Asn Ser Ser Thr Ile Thr Gln Ala Cys Pro Lys Val Thr Phe Asp
 195 200 205
 Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys
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 Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser
 225 230 235 240
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 725 730 735

61

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Lys Leu Phe Asp Thr Ile Ala Ile Ala Val Ala Glu Gly Thr Asp Arg
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Ile Arg Ile Arg Gln Gly Phe Glu Ala Ala Leu Gln
835 840

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(21) International Application Number: **PCT/US01/21701**

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(26) Publication Language: English

(30) Priority Data:
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Published:

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Filed on 7 July 2000 (07.07.2000)

(88) Date of publication of the international search report:
29 August 2002

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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WO 02/003917 A3

(54) Title: ALPHAVIRUS VECTORS AND VIROSOMES WITH MODIFIED HIV GENES FOR USE AS VACCINES

(57) Abstract: The present invention provides methods and compositions comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit integrase, RNase H and/or reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/21701

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/12
US CL : 424/199.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/ 93.2; 435/69.1, 173.3, 199.1, 235.1, 236, 239, 320.1, 325; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BAROUCH et al. Augmentation of immune responses to HIV-1 and simian immunodeficiency virus DNA vaccines by IL-2/Ig plasmid administration in rhesus monkeys. Proceedings of the National Academy of Science, April 2000, Vol. 97, No. 8, pp. 4192-4197, see figure 1.	1, 21 -----
---		2, 3, 22, 23
Y	US 5,792,462 A (JOHNSTON et al.) 11 Aug. 1998 (11.08.1998), column 2 lines 14-38 and examples 1-4.	41, 42 -----
---		2, 3, 22, 23, 44, 70, 71, 74
Y	SYKES et al. Genetic Live Vaccines Mimic the Antigenicity But Not Pathogenicity of Live Viruses. DNA and Cell Biology, 1999, Vol. 18, No. 7, pp. 521-531, see	1, 2, 3, 21, 22, 23
X	US 6,156,558 A (JOHNSTON et al.) 5 Dec. 2000 (05.12.2000), see claims.	41, 42 -----
---		2, 3, 22, 23, 44, 70, 71, 74
Y		55, 57
A	FEYZI et al. Structural Requirement of Heparin Sulfate for Interaction with Herpes Simplex Virus Type 1 Virions and Isolated Glycoprotein C*. The Journal of Biological Chemistry, 1997, Vol. 272, No. 40, pp. 24850-24857, see methods.	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

02 November 2001 (02.11.2001)

Date of mailing of the international search report

12 JUN 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
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Washington, D.C. 20231
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Authorized officer

Ulrike Winkler, Ph.D.

Telephone No. 703-308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/21701

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 6,146,874 A (ZOLOTUKHIN et al.) 14 Nov. 2000 (14.11.2000), see figure 1.	55, 57

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/21701

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-3,21-23,41,42,44,55,57,70,71 and 74

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest



The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/21701

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1, 21, drawn to a nucleic acid composition comprising two or more HIV nucleic acids.

Group 2, claim(s) 2, 3, 22 and 23 drawn to an alphavirus replicon particle.

Group 3 claim(s) 4-10, 24-30 drawn to a method of making the alphavirus replicon particle.

Group 4, claim(s) 11, 31, drawn to a method of inducing an HIV immune response using nucleic acids.

Group 5, claim(s) 12-15 and 32-35 drawn to a method of inducing an HIV immune response using an alphavirus particle.

Group 6, claim(s) 16, 36 drawn to a method of treatment or prevention of HIV using a nucleic acid composition.

Group 7, claim(s) 17-20 and 37-40 drawn to a method of treatment or prevention of HIV using a alphavirus particle.

Group 8, claim(s) 41, 42, and 44, drawn to an alphavirus replicon virosome.

Group 9, claim(s) 43, drawn to method of producing an alphavirus virosome.

Group 10, claim(s) 45, drawn to method inducing an immune response using a virosome.

Group 11, claim(s) 46, drawn to a method treating or preventing HIV infection using a virosome.

Group 12, claim(s) 47 and 48, drawn to a composition comprising an alphavirus replicon virosome comprising HIV genes.

Group 13 claim(s) 49 and 50 drawn to a method of making the alphavirus replicon particle comprising HIV genes.

Group 14 claim(s) 51 and 52 drawn to a method of eliciting an immune response to HIV utilizing an alphavirus virosome.

Group 15 claim(s) 53 and 54 drawn to a method preventing or treating HIV infection using an alphavirus virosome.

Group 16 claim(s) 55 and 57 drawn to a composition comprising a heparin affinity purified alpha virus replicon particle.

Group 17 claim(s) 56 drawn to a method of making the alphavirus replicon particle utilizing heparin affinity chromatography.

Group 18 claim(s) 58-60 drawn to a method of making VRP for a vaccine.

Group 19 claim(s) 61-64 drawn to an isolated *pol* gene product.

Group 20 claim(s) 65, 68 and 69 drawn to an alphavirus particle comprising a *pol* gene product.

Group 21 claim(s) 66 and 67 drawn to a method of making the alphavirus replicon particle comprising the *pol* gene product.

Group 22 claim(s) 70, 71 and 74 drawn to a method of inducing an immune response with the alphavirus replicon particle comprising the *pol* gene product.

Group 23 claim(s) 72, 73 and 75 drawn to a method of treating or preventing disease with the alphavirus replicon particle comprising the *pol* gene product.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/21701

The inventions listed as Groups 1-23 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-23 appears to be the nucleic acid composition set out in claim 1. The nucleic acid composition requires two or more isolated nucleic acid comprising either *env*, *gag* or *pol* gene products. Barouch et al. (PNAS April 2000) disclose the immunization of mice using a composition of two plasmids encoding *gag* and *env* genes (see figure 1). This composition is injected into mice and the immune response is measured after several weeks indicating that the gene products encode immunogenic fragments. Therefore, the technical feature linking the inventions of groups 1-23 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of group 1 is considered to be the nucleic acid composition.

The special technical feature of group 2 is considered to be an alphavirus replicon particle.

The special technical feature of group 3 is considered to be a method of making an alphavirus replicon particle.

The special technical feature of group 4 is a method of inducing an immune response to HIV using nucleic acids.

The special technical feature of group 5 is considered to be a method of inducing an immune response to HIV using an alphavirus replicon particle.

The special technical feature of group 6 is considered to be a method of treating or preventing HIV using nucleic acids.

The special technical feature of group 7 is considered to be a method of treating or preventing HIV using an alphavirus replicon particle.

The special technical feature of group 8 is considered to be an alphavirus replicon virosome.

The special technical feature of group 9 is considered to be a method of making an alphavirus replicon virosome.

The special technical feature of group 10 is considered to be a method inducing an immune response utilizing a virosome.

The special technical feature of group 11 is considered to be a method of treating or preventing HIV using a virosome.

The special technical feature of group 12 is considered to be a composition comprising an alphavirus replicon virosome comprising HIV genes.

The special technical feature of group 13 is considered to be a method of making alphavirus replicon virosome comprising HIV genes.

The special technical feature of group 14 is considered to be a method of eliciting an immune response to HIV using an alphavirus replicon virosome.

The special technical feature of group 15 is considered to be a method of treating or preventing HIV using an alphavirus replicon virosome.

The special technical feature of group 16 is considered to be a composition comprising a heparin affinity purified alpha virus replicon particle.

The special technical feature of group 17 is considered to be a method of making a heparin affinity purified alphavirus replicon particle.

The special technical feature of group 18 is considered to be a method of making VRP for a vaccine.

The special technical feature of group 19 is considered to be a the isolated *pol* gene product.

The special technical feature of group 20 is considered to be an alphavirus particle comprising the *pol* gene product.

The special technical feature of group 21 is considered to be a method of making the alphavirus particle comprising the *pol* gene product.

The special technical feature of group 22 is considered to be a method of inducing an immune response with an alphavirus replicon particle comprising the *pol* gene product.

INTERNATIONAL SEARCH REPORT

International application No.

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The special technical feature of group 23 is considered to be a method of treating or preventing disease with an alphavirus replicon particle comprising the pol gene product.

Accordingly, groups 1-23 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, DERWENT, MEDLINE, STN-BIOSIS

Togaviridae, alphavirus, flavivirus, Venezuelan equine encephalitis virus, VEE, replicon, purification, heparin, heparin affinity